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Abstract Book

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European Journal of Endocrinology Prize Lecture

The *European Journal of Endocrinology* Prize is awarded to a candidate who has significantly contributed to the advancement of knowledge in the field of endocrinology through publication. This year's recipient is Professor Claes Ohlsson. The prize will be presented as part of the ECE 2008 opening ceremony where Professor Ohlsson will deliver his lecture. Professor Ohlsson will also write a review article based on this lecture to be published in the *European Journal of Endocrinology*. Further information can be found at http://www.euro-endo.org/about/about_prizes.htm

Claes Ohlsson, Sweden

Claes Ohlsson is professor at the Centre for Bone Research, Institute of Internal Medicine at the Sahlgrenska Academy in Göteborg, Sweden (2000-). He received his MD in 1990 and his PhD in 1993 both from Göteborg University. He has a board certificate as physician in Clinical Pharmacology and was a post graduate research fellow at NIH, Bethesda in 1996–1997. Dr Ohlsson has made several contributions to the field of osteoporosis with a special focus on *hormonal regulation of bone growth and metabolism*. His research on osteoporosis has a translational profile, combining cell and molecular biology with experimentation on animals and human tissue from patients, as well as epidemiological methods.

Dr Ohlsson has been working in the field of bone and mineral metabolism since 1990. His initial research aimed at increasing the understanding of the molecular and cellular mechanisms for the effects of growth hormone and IGF-I on skeletal growth and adult bone metabolism. His current focus is to increase the understanding of the mechanisms of action of sex steroids on bone metabolism. To study this, his research group develops and analyzes several different transgenic mouse models, including tissue specific over expression and inactivation of key genes involved in sex steroid action.

In addition, he has established unique well characterized longitudinally followed population-based cohorts, investigated not only by the dual energy X-ray absorptiometry technique but also with the more informative computer tomography technique. Using these cohorts, his research group has elucidated the impact of environmental and genetic factors on peak bone mass and age-dependent bone loss.

He is currently principal investigator of a research group consisting of 20 post docs, PhD. students and technicians supported by funding from the European Union, the Swedish Research Council and the Swedish Strategic Foundation. Dr Ohlsson has received the 'Jubileums' award from the Swedish Medical Association, the 'Fernström' award and the 'INGVAR' award for young successful researcher by the Swedish Strategic Foundation. In 2006, he received the Scandinavian SALUS-ansvar price in Medicine. He has published more than 200 original articles in peer-reviewed journals and has been invited speaker to 45 international meetings including the major meetings in Endocrinology and Osteoporosis.

Sex steroids in the regulation of bone metabolism in men

Claes Ohlsson, Division of Endocrinology, Department of Internal Medicine, Sahlgrenska University Hospital, Goteborg, Sweden

Osteoporosis-related fractures constitute a major health concern not only in women but also in men. The relative contribution of estrogens and androgens for the male skeleton remains unclear. Most epidemiological studies demonstrate that serum estradiol is a stronger predictor of bone mineral density than serum testosterone. However, conflicting results have been presented regarding the impact of serum sex steroids for fracture risk in men, probably because previous studies have been underpowered and have analyzed the serum sex steroid levels using immunoassay-based techniques with a questionable specificity at lower concentrations. We recently showed that elderly men with low serum estradiol have an increased risk of fractures in the large population-based MrOS Sweden study, with serum sex steroids analyzed by the specific gas chromatography–mass spectrometry technique. In contrast, serum testosterone was not an independent predictor of fracture risk.

There are two main sources of sex steroids in elderly men, the testicles and the adrenals. Interestingly, we found that low serum DHEA was related to fracture risk independently of serum sex steroids in the MrOS Sweden study, indicating that adrenal-derived DHEA, which is locally converted to estradiol and/or testosterone, has an impact on fracture risk.

Experiments using mice with inactivated sex steroid receptors demonstrated that both activation of the estrogen receptor (ER)α and activation of the androgen receptor (AR) result in a stimulatory effect on the cancellous bone mass in males. ERβ was of no importance for the skeleton in male mice while it modulated the ERα-action on cancellous bone in females. *In vitro* studies demonstrated that the G-protein coupled receptor GPR30 is a functional ER. Our recent *in vivo* analyses of GPR30-inactivated mice revealed no function of GPR30 for cancellous bone mass but it is involved in estrogen-mediated insulin secretion and modulates longitudinal bone growth.

Geoffrey Harris Prize Lecture

This prestigious prize is intended for established workers in the field of basic or clinical neuroendocrinology, and is generously support by Ipsen. This year's recipient is Professor George P Chrousos. The prize will be presented as part of the ECE 2008 opening ceremony where Professor Chrousos will deliver his lecture. Professor Chrousos will also give two other lectures at future ESE scientific meetings. Further information can be found at http://www.euro-endo.org/about/about_prizes.htm

George P Chrousos, Greece

Dr George P Chrousos is Professor and Chairman of the Department of Pediatrics at the University of Athens School of Medicine, Athens, Greece, and former Chief of the Pediatric and Reproductive Endocrinology Branch of the National Institute of Child Health and Human Development (NICHD), National Institutes of Health (NIH), Bethesda, Maryland. Prof Chrousos has made major contributions to Neuroendocrinology. He has worked on the principal stress-responsive hormonal neuro-axis, the hypothalamic-pituitary-adrenal (HPA) axis for over 25 years, and has helped in the elucidation of fundamental physiological and molecular mechanisms, through which this axis regulates growth, development, reproduction, sleep and successful behavioral, metabolic, and immunologic adaptation. His studies on the role of neuroendocrine dysregulation in disease have been equally influential. They have illuminated pathophysiological mechanisms, improved diagnosis and treatment, and refined the clinical approaches employed in managing patients with illnesses that span a range of medical disciplines, including Medicine and Pediatrics, Endocrinology, Psychiatry, Rheumatology, Allergy, Surgery, Oncology and Reproductive Medicine. Dr Chrousos has written over 1000 scientific papers and his work has been cited in more than 40 000 other scientific articles, an irrefutable testimony to the importance and influence of his research. He is one of the most cited physician scientists (*ISI highly cited*) both in *Clinical Medicine* and in *Biology and Biochemistry*. His work has educated a broad community of physicians and scientists around the world. As a mentor, he has fostered the careers of many young physicians and scientists, several of whom are now professors and chairpersons in Europe, the United States, Asia, Australia, and Latin America. An outstanding teacher, he has had many visiting professorships and given prestigious lectures throughout the world. Dr Chrousos has received numerous national and international awards for his work, including election to the prestigious American Society of Clinical Investigation and the Association of American Physicians. He was inducted as a master of both the American College of Endocrinology and the American College of Physicians. He is president of the European Society of Clinical Investigation.

From Goeffrey Harris's hypothalamic principle to a unified theory of stress and stress system disorders

George P Chrousos, University of Athens, Athens, Greece

The existence of the principle of hypothalamic hypophysiotropic factors, predicted so prophetically by G W Harris in the 1940's, has been confirmed by irrefutable evidence. This principle is central to the survival of complex organisms as both individuals and species. Life exists through maintenance of a complex dynamic equilibrium, or homeostasis, that is constantly challenged by intrinsic or extrinsic adverse forces, or stressors. Thus, stress is defined as a state of threatened homeostasis that is re-established by a complex repertoire of physiologic and behavioral adaptive responses of the organism. Neuroendocrine hormones play crucial roles in the coordination of both basal and threatened homeostasis and mediate the pathogenesis of dyshomeostatic disease states. The stress response is subserved by the stress system, which is located both in the central nervous system and the periphery. The principal central effectors of the stress system, which are highly interlinked, include the hypothalamic corticotropin-releasing hormone (CRH), arginine vasopressin, and proopiomelanocortin-derived peptides (alpha-melanocyte-stimulating hormone and beta-endorphin), and the brainstem locus caeruleus (arousal) and central autonomic norepinephrine. The principal peripheral effectors are the glucocorticoids, the catecholamines norepinephrine and epinephrine and, interestingly, peripheral (immune) CRH and interleukin-6. The targets of these effectors are in the brain, including the reward, fear and executive systems and the wake/sleep centers, the growth, thyroid and reproductive axes, as well as the gastrointestinal, cardiorespiratory, metabolic, and immune systems. Appropriate basal activity and responsiveness of the stress system to stressors is a crucial prerequisite for a sense of well-being, adequate performance of tasks, and positive social interactions. By contrast, inappropriate basal activity and responsiveness of the stress system may impair growth, development and body composition, and may account for many endocrine, metabolic, autoimmune, allergic and behavioral disorders. The development and severity of these conditions primarily depend on the genetic and constitutional vulnerability of the individual, the exposure to critical period or concurrent adverse or protective environmental factors, and the timing, size and duration of the stressor(s). Prenatal life, infancy, childhood and adolescence are critical periods characterized by increased vulnerability to stressors.

Overproduction of CRH and stress system abnormalities are observed in behavioral/psychiatric disorders, such as hypothalamic oligo-amenorrhea, obligate athletisism, depression, anxiety, post-traumatic stress disorder, eating disorders and addiction. Investigations of CRH type 1 receptor (CRHR1) nonpeptide antagonists, such as the prototype Antalarmin, suggest therapeutic potential for treatment of these and other neuropsychiatric entities. Overproduction of CRH in the brain and the periphery and disruption of the hypothalamic–pituitary–adrenal (HPA) axis and the arousal and sympathetic systems, on the other hand, are also found in 'somatic' disorders, suggesting that CRHR1 antagonists may also be efficacious in treating common stress-related diseases, such as obesity/metabolic syndrome and essential hypertension, that are often associated with subtle but chronic hyperactivity of the stress system, representing central dysregulation of CRH and norepinephrine, and leading to cardiovascular disease. Furthermore, autonomic dysregulation is a prominent feature of common gastrointestinal disorders, such as irritable bowel syndrome and peptic ulcer disease. As CRH modulates bowel and gastric activity both directly and through the autonomic nervous system and influences centrally the processing of viscerosensory and visceromotor neural signals, it is not surprising that preclinical and clinical evidence suggests a central role of CRH in the pathophysiology of these stress-related gastrointestinal disorders as well. Neuroendocrine, autonomic and immune aberrations are also present in chronic inflammatory/autoimmune and allergic diseases, as well as in the chronic fatigue and fibromyalgia syndromes, with considerable evidence linking low CRH activity to the observed abnormalities. Similar low CRH activity has been implicated in atypical, seasonal depression, postpartum blues/depression and the late luteal dysphoric disorder and climacteric depression.

Plenary Lectures

Estrogens and cardiovascular disease

PL1

Estrogens and cardiovascular disease

Michael Mendelsohn
Tufts Medical Center, Boston, Massachusetts, USA.

In the 1990s, we learned that the same estrogen receptors that mediate hormonal effects in reproductive tissues also function in the heart and blood vessels, where they are required for normal cardiovascular physiology and for estrogen-mediated protection against vascular injury and atherosclerosis. Great progress has been made in the past decade in understanding the importance of ERα and ERβ in cardiovascular physiology and disease. In this presentation, data regarding the specific role of ERs in cardiovascular physiology and pathophysiology will be reviewed and biological explanations for the WHI-generated controversy and confusion regarding hormone replacement therapy (HRT) will be highlighted. Newer clinical evidence that more firmly supports the beneficial cardiovascular effects of HRT for menopausal women also will be reviewed and explained in the context of basic science and animal studies from the past 15 years that clearly support a central protective role for estrogen and ERs in cardiovascular disease. Molecular and cellular explanations for the controversy that arose from the Women's Health Initiative (WHI) trials of the cardiovascular effects of HRT on CVD will be discussed and differences in the underlying vascular biology that exists between younger and older menopausal women that support the importance of timing of HRT initiation will be reviewed. Several newer concepts in sex steroid hormone receptor action that have important implications for cardiovascular physiology and disease also will be discussed. These include the role of receptor co-regulatory proteins in cardiovascular cell biology, rapid ('non-genomic') activation of vascular endothelial cells by estrogen and ERs, ligand-independent ER activation in vascular cells, gender differences in regulation of metabolic pathways important to cardiovascular diseases by other NHR, and genetic ER variants associated with altered cardiovascular risk in both sexes. The ways in which these newer pathways can add substantial combinatorial complexity to the physiological effects of estrogen in target cardiovascular tissues will be discussed.

Let's get older-lessons to learn from centenarians

PL2

Why did Moses live to be 120?

Nir Barzilai
Albert Einstein College of Medicine, Bronx, New York, USA.

Aging is associated with an increase in all the components of the metabolic syndrome and decline in the endocrine axis. We asked if healthy centenarians are over-represented with phenotype and genotype for exceptional longevity that protects them from the endocrine causes of aging. For that, we recruited a genetically homogenous population of unrelated ashkenazi jews with exceptional longevity ($n \sim 450$). To validate the genetic and physiological findings we also recruited the offspring of these subjects with exceptional longevity ($n \sim 400$). We assessed their clinical (NCEPIII guidelines), their metabolic (lipoproteins, insulin), and endocrine (IGF-1 and its bonding proteins, TSH) phenotype. We used a several method to genotype numerous SNPs in promoters, exons or introns of genes associated with this phenotype, and a method to discover new mutations. We show that centenarians have a unique liporotein profile that is inherited by their offspring. We demonstrate 3 genotypes in cholesterol ester transfer protein (CETP), apo-lipoprotein c-3 (APOC3), and adiponectin (ADIPOQ) that monotonically increase between ages 60–110 in unrelated individuals, and are over represented by 2–3 folds in the oldest old group, suggesting that they may be necessary to assure exceptional survival. Furthermore, we show that these genotypes are associated with changes in their protein plasma levels and out come related to the metabolic syndrome. We also demonstrate the role of IGF-1 receptor mutation in exceptional longevity and of length of telomeres in the metabolic syndrome. We will support the notion that exceptional longevity is associated with enrichment in the several genotypes, and significant measurable phenotype. These genotypes and their associated phenotype may play a role in conferring survival to exceptionally old age by providing protection from the metabolic syndrome and other endocrine manifestation of aging.

The red wine hypothesis: resveratrol and human metabolism

PL3

Central regulation of appetite and body weight: the role of peripheral signals

Jens Brüning
Institute for Genetics, Cologne, Germany.

The ever-growing obesity epidemic puts an enormous burden on our societies. The need for the development of novel therapeutic interventions necessitates the exact understanding of the underlying regulatory principles in energy homeostasis. Over the last 10 years, the hormonal and nutritional factors communicating the energy state of the body to the central nervous system to adapt caloric intake and energy expenditure have rapidly extended our understanding on the mechanisms controlling energy homeostasis. The presentation will focus on insights into the primary neuronal target sites of such signals as well as molecular signaling mechanisms involved mainly obtained through the use of mice with conditional inactivation of signaling components in specific neuronal populations.

Berthold lecture of the German Endocrine Society

PL4

Abstract unavailable

Too little and too much insulin – lessons to learn from newborns

PL5

Too little and too much insulin: lessons from newborns

Andrew Hattersley
Penisula Medical School, Exeter, UK.

Insulin is a crucial growth factor *in utero* as well as the key post natal determinant of blood glucose. Mutations in beta-cell genes altering insulin secretion therefore present with both altered birth weight and also hyper or hypoglycaemia. Recent advances in the genetics of neonatal diabetes and neonatal hyperinsulinism give key insights to beta-cell physiology as well as offering improved clinical management.

In the genes encoding key beta-cell proteins Kir6.2, SUR1 and glucokinase different mutations will result in both activating and inactivating mutations and result in the opposing phenotypes of hypo and hyper glycaemia. Recently, we showed mutations in HNF4alpha the same loss of function mutations result in both transient neonatal hypoglycaemia and also latter beta-cell failure and diabetes.

There have been major advances in the genetics of neonatal diabetes and a molecular genetic diagnosis is now possible most patients with transient or permanent neonatal diabetes. In transient neonatal diabetes (TNDM) is now possible to give a diagnosis in over 95% of patients. The commonest cause are abnormalities of imprinting in the region of the ZAC gene on 6q (71%) the majority of other patients either have a mutation in SUR1 (14%) or Kir6.2 (12%). K_{ATP} mutations like ZAC anomalies that cause TNDM may also relapse with permanent diabetes outside the neonatal period.

In permanent neonatal diabetes (PNDM), the commonest cause is Kir6.2 mutations (30%) but SUR1and insulin mutations that may present as dominant or recessive mutations are found in 12 and 14% of cases, respectively. Neurological features which account for approximately 20% of Kir6.2 mutations are seen in $<5\%$ in SUR1 patients and are not a feature of insulin mutations.

The major reason that genetics have been important in neonatal diabetes is because it had altered treatment. Patients with SUR1 and Kir6.2 mutations, even if insulin dependent, can improve control by replacing insulin injections with sulphonylurea tablets.

Neonatal diabetes is now an area where a molecular genetic diagnosis is not a luxury but a necessity. A molecular diagnostic service is provided by centres throughout the world including our own on www.diabetesgenes.org and is having a positive impact on the care of patients with neonatal diabetes.

Graves' ophthalmopathy – problems solved and new questions to be answered

PL6

Graves' ophthalmopathy: problems solved and new questions to be answered

Wilmar M Wiersinga
Academic Medical Center, Amsterdam, The Netherlands.

Graves' ophthalmopathy (GO) remains the most enigmatic presentation of thyroid autoimmunity. Although many issues regarding its pathogenesis and management have been solved, they are still outnumbered by unresolved open questions.

Pathogenesis

Orbital fibroblasts (OF) have been recognized as the primary target cells of the autoimmune attack. Cytokine-induced excessive secretion of glycosaminoglycans and differentiation of a subset of OF into adipocytes cause swelling of extraocular muscles and orbital fat, explaining in a mechanistic sense symptoms and signs of GO. The nature of the autoantigen on OF remains elusive, although the TSH receptor is favoured over others (like the IGF-1 receptor). Attempts to develop a suitable experimental animal model are still unsuccessful.

Clinical presentation

Graves' hyperthyroidism (GH) and GO apparently belong to the same disease entity, as the majority of GH patients have subclinical GO and euthyroid GO patients have TSH receptor autoantibodies. Why not all GH patients develop overt GO remains (apart from smoking) poorly understood; also why not all euthyroid GO patients develop overt GH. Whereas unilateral GO frequently evolves into bilateral GO many cases remain strictly unilateral for unknown reasons. Most patients have increased muscle and fat volumes, but some have only increased muscle or fat volume, probably indicating various phenotypes with different immunopathogenesis.

Management

It matters for the eyes whether the patient is euthyroid and how euthyroidism is restored, although controversy continues on the benefit of total thyroid ablation. There is broad agreement that immunosuppression is indicated only in active GO, and that iv pulses of methylprednisolone are most effective. Anticytokine treatment (rituximab) is promising, but in its early stages. Combination treatment might be more efficaceous than monotherapy.

Prevention

Discontinuation of smoking constitutes primary, secondary and tertiary prevention. Determinants of progression from mild to more severe GO are incompletely understood, and hamper effective intervention (with selenium?) to halt progression.

When and why do we wake up – the endocrine regulation of sleep in humans

PL7

Decreased sleep duration and quality: novel risk factors for obesity and diabetes

Eve Van Cauter
The University of Chicago, Chicago, Illinois, USA.

Sleep curtailment has become a common behavior in industrialized countries. Simultaneously, the aging of the population is associated with an increased prevalence of sleep disturbances. These trends for shorter sleep duration and poorer sleep quality have developed over the same time period as the dramatic increase in the prevalence of obesity and diabetes. There is recent evidence to indicate that chronic partial sleep loss and decreased sleep quality may increase the risk of obesity and diabetes. Studies in healthy volunteers have shown that sleep restriction (4–6 h bedtimes) is associated with an adverse impact on glucose homeostasis. Insulin sensitivity decreases rapidly and markedly without adequate compensation in beta cell function, resulting in an elevated risk of diabetes. Multiple factors appear to mediate this adverse impact of sleep loss, including increased sympathetic nervous activity, decreased brain glucose uptake and elevated evening cortisol levels. Reduced sleep quality, without change in sleep duration, is also associated with an increased risk of diabetes. Indeed, selective suppression of slow-wave sleep, a highly heritable trait, rapidly results in a marked reduction in insulin sensitivity and disposition index. Prospective epidemiologic studies in children and adults are consistent with a role for sleep disturbances in the increased risk of diabetes. Sleep curtailment is also associated with a dysregulation of the neuroendocrine control of appetite. Under conditions of controlled caloric intake and energy expenditure, there is a negative relationship between leptin levels and sleep duration. In a randomized cross-over design study (2 days of 4-h versus 8-h bedtimes), leptin levels were decreased and ghrelin levels increased during the short sleep condition, and the change in the ghrelin to leptin ratio was strongly correlated with increased hunger. Thus, sleep loss may alter the ability of leptin and ghrelin to accurately signal caloric need. Consistent with the laboratory evidence, epidemiologic studies have shown an association between short sleep and higher BMI after controlling for a variety of possible confounders. Taken together, the current evidence suggest that chronic partial sleep curtailment, a novel behavior that appears to have developed with the advent of the 24-h society, and reduced sleep quality may be involved in the current epidemic of obesity and diabetes.

Central regulation of appetite and body weight – the role of peripheral signals

PL8

A cofactor network that controls PGC-1α activity and energy homeostasis

Johan Auwerx
Institut de Génétique et de Biologie Moléculaire et Cellulaire and Institut Clinique de la Souris, Illkirch, France.

Dysfunctional mitochondrial oxidative phosphorylation and diminished aerobic capacity are associated with metabolic, cardiovascular and neurodegenerative diseases that eventually alter life span. We will discuss two distinct signalling pathways to control energy expenditure that converge on the coactivator PGC-1α. In a first study we focussed on the polyphenol resveratrol, an activator of the class III HDAC SIRT1, the mammalian Sir2 homolog. Resveratrol significantly increased aerobic capacity as evidenced by the doubling of time that mice run on a treadmill and by the increased oxidative capacity in muscle fibers *ex vivo*. This enhanced muscle performance was associated with an increase in expression of genes encoding for oxidative phosphorylation and mitochondrial biogenesis. These molecular events were largely explained by the resveratrol-mediated increase in PGC-1α activity, both through an increase in its expression level and a decrease in PGC-1α acetylation, fitting with the fact that resveratrol activates SIRT1. Importantly, the improved mitochondrial activity induced by resveratrol treatment protected mice against diet induced obesity and insulin resistance. In a second study, we characterized the role of SRC-3 in energy metabolism. SRC-1 and -2, two members of the p160 cofactor family were previously shown by us to affect energy homeostasis. Differently from SRC-1 and -2 KO mice, SRC-$3^{-/-}$ animals weigh less under basal conditions, an effect which is accentuated by a high fat diet. This lean phenotype of SRC-$3^{-/-}$ mice is associated with increased energy expenditure in BAT and skeletal muscle subsequent to enhanced mitochondrial activity. The effect on energy expenditure in the SRC-$3^{-/-}$ mice is dependent on the increase in both PGC-1α expression and activity, subsequent to its decreased acetylation. In combination, these data suggest that SRC-3 and SIRT-1 are critical links in a complex cofactor network that is governed by PGC-1α and that controls energy homeostasis. This work opens up new perspectives for therapeutic and preventive strategies for metabolic diseases.

Symposia

The trick is the combination – S1

S1.1

Treatment of hyperthyroidism: block and replace versus titration

Luigi Bartalena
University of Insubria, Varese, Italy.

The ideal treatment of hyperthyroidism due to Graves' disease (GD), an autoimmune disorder ultimately caused by TSH-receptor antibody, would consist of the elimination of disease triggers. Because this is not feasible, current management relies on either thyroid ablation (thyroidectomy and/or radioiodine) inevitably bound to subsequent hypothyroidism, or a conservative approach using antithyroid drug (ATD) treatment. The latter is associated with a high rate (about 40–50%) of relapse of hyperthyroidism. Commonly used ATDs (thionamides) mostly act by inhibiting thyroid hormone synthesis, although (direct or indirect) immunosuppressive actions are also postulated. Two different ATD regimens are in common use for GD: i) Titration method; ii) Block-and-replace method. In the titration method, the usual starting dose is 15–30 mg/day methimazole (or equivalent doses of other thionamides); further to periodic thyroid status assessment, daily dose is tapered down to the lowest effective dose (avoiding both hyper- and hypothyroidism). Thyroid function tests are checked every 4–6 weeks for the first 4–6 months, then every 3–4 months until treatment is stopped after 18–24 months. The block-and-replace method uses persistently high ATD doses in association with L-thyroxine replacement to avoid hypothyroidism; treatment lasts 6 months. This method has advantages and disadvantages over the titration method. Higher doses of ATDs may have a greater immunosuppressive action useful for a permanent remission of hyperthyroidism, but this putative effect remains to be demonstrated. Avoidance of hypothyroidism or 'escape' of hyperthyroidism seems easier than with the titration method; treatment is shorter, and the number of visits lower. On the other hand, the much higher number of tablets taken every day may create problems of poor compliance. The block-and-replace method should not be used during pregnancy. A recent systematic review (Abraham *et al*., *Eur J Endocrinol* 2005 **153** 489–498) showed that the block-and-replace method has no advantages in terms of permanent remission of hyperthyroidism, while the prolonged use of high ATD doses may bear a higher risk of side effects. The latter conclusion has recently been questioned (Razvi *et al*., *Eur J Endocrinol* 2006 **154** 783–786), but in the absence of powered controlled trials comparing the two regimens, this author feels that the titration method is preferable.

S1.2

The combination of GH/IGF-I makes the difference!

Joseph Janssen
Erasmus MC, Rotterdam, The Netherlands.

Growth hormone (GH) is the primary regulator of insulin-like growth factor-I (IGF-I) production in a wide variety of tissues. After secretion by the pituitary GH, GH is transported to the liver and stimulates IGF-I production in the liver. The IGF-I produced in the liver accounts for most of the IGF-I in the circulation. The circulating IGF-I will have effects on extra hepatic tissues as the heart, lung, muscles and kidney. Circulating IGF-I feeds back at the level of the pituitary gland and the hypothalamus to inhibit growth hormone secretion. GH may bypass the liver and directly stimulate IGF-I production in other target organs. In this way GH stimulates locally IGF-I production in an autocrine or paracrine fashion. In addition, in many tissues IGF-I can be locally formed in autocrine and paracrine fashion and induce effects independently from GH. In the circulation IGF-I is almost for 100% bound to the so-called insulin-like binding proteins, the IGFBPs. The IGFBPs have many functions: they act as transport proteins, they prolong half-life of IGF-I in the circulation. Another function of the IGFBPs is the modulating of IGF-I activity. In some situations they will stimulate IGF-I effects, but in some other circumstances they will inhibit/prevent IGF-I actions, and finally by the binding of IGF-I to the IGFBPs the body has a manner to deliver IGF-I in a tissue- and cell-specific way.Why should combination of GH and IGF-I be more effective than GH alone or IGF-I alone? I will give some arguments: 1) Clearance of IGF-I may be markedly altered by the (co)administration of GH, Since GH administration stabilizes the formation of the ternary IGF-binding complex, it potentially will provide sustained action of IGF-I. 2) Higher serum IGF-I levels are achieved with a combination treatment of GH and IGF-I than with GH treatment alone or IGF-I alone. 3) The combination GH and IGF-I counteracts disadvantageous effects on glucose metabolism of either GH alone or IGF-I alone and is synergistic with respect to lipolysis and proteolysis. 4) GH may exert direct actions on tissues independently from IGF-I. 5) The combination of GH and IGF-I may be more effective in improving tissue IGF-I levels, and this latter effect may be more important than increasing serum IGF-I concentrations. In conclusion, the combination of GH and IGF-I may be more effectively improving tissue IGF-I levels and may have substantially more anabolic actions and a better balanced glycaemic control than GH alone or IGF-I alone.

S1.3

Medical treatment of acromegaly: dual blockade with a somatostatin analog and Pegvisomant

Jens Jorgensen
Aarhus University Hospital, Aarhus, Denmark.

Transsphenoidal surgery is preferred as primary therapy for acromegaly, but cure or acceptable disease control with this modality is obtained in $<60\%$, and in some cases surgery is not eligible. Second-line treatment with long-acting somatostatin analogues (SA) is successful in $\sim 60\%$. This treatment offers tumor shrinkage in addition to lowering of GH and IGF-I in most patients. A potential concern is impairment of glucose tolerance due to the concomitant suppression of insulin secretion. Pegvisomant is a specific GH antagonist, which binds to and blocks the GH receptor. This compound is very effective in providing IGF-I nomalisation and symptom relief in more than 90%. The treatment results in increased serum levels of endogenous GH, which probably reflects increased secretion and reduced clearance. This could theoretically lead to increased growth of remnant GH secreting tumor tissue.
A combination of a SA analogue and pegvisomant is potentially attractive by targeting the disease at two different levels: 1) suppression of tumor activity, and 2) peripheral blockade of GH action. Indeed, a number of trials confirm that this principle is feasible, in particular it offers superior IGF-I lowering and improved glucose tolerance as compared to SA mono-therapy in patients who are partially resistant to SA. There is also evidence to suggest that combination therapy may be cost-neutral by reducing the demand of pegvisomant.
Conclusions
1) Disease control by medical therapy is now obtainable in almost all patients with acromegaly, 2) Long term data on the effects and side effects of combination therapy are still needed and 3) Should combination therapy be first choice to all patients who do not respond adequately to SA?

S1.4

The trick is the combination

Wiebke Arlt
Birmingham University, Birmingham, UK.

This lecture will address the timely topic of androgen replacement in women, which according to consensus guidelines should only be carried out if adequate estrogenization is provided. Female androgens either derive from direct ovarian production or from peripheral conversion of the adrenal sex steroid precursor, dehydroepiandrosterone, towards active androgens. Therefore, loss of adrenal or ovarian function, caused by Addison's disease or consequent to bilateral oophorectomy, results in severe androgen deficiency, clinically often associated with a loss of libido and energy. Importantly, physiological menopause per se does not cause androgen deficiency, as androgen synthesis in the ovaries may persist despite the decline in estrogen production. However, the definition of female androgen deficiency, as recently provided by 2002 Princeton consensus statement, is not precise enough and may lead to over-diagnosis due to the high prevalence of its diagnostic criteria: androgen levels below or within the lower quartile of the normal range and concurrent sexual dysfunction. On the other hand, the Endocrine Society USA guidelines published in 2006 and advising against all androgen replacement in women, is of no better help. Currenlty, androgen treatment should be reserved for women with severe androgen deficiency due to an established cause (mostly adrenal insufficiency, bilateral oophorectomy) and matching clinical signs and symptoms. Replacement options include transdermal testosterone administration or dehydroepiandrosterone treatment, both of which have been shown to result in significant improvements, in particular in libido and mood, while effects on body composition and muscular function are not well documented. It is important to keep in mind that the number of randomized controlled trials is still limited and we need to learn more about benefits and risks.

TGFβ superfamily – S2

S2.1

BMP to the bone

Peter ten Dijke
Leiden University Medical Center, Leiden, The Netherlands.

Bone morphogenetic proteins (BMPs) are multifunctional proteins that regulate the fate of different cell types, including mesenchymal. BMPs promote the differentiation of mesenchymal cells into functional osteoblasts. Like other

members of the transforming growth factor-β superfamily, BMPs elicit their cellular effects via specific types I and II serine/threonine receptors. The activated BMP type I receptor phosphorylates specific receptor-regulated (R)-Smad proteins, which assemble into heteromeric complexes with common partner (Co)-Smad4. Heteromeric Smad complexes efficiently translocate into the nucleus, where they regulate the transcription of target genes. Inhibitors of differentiation (Id) are genes that are specifically induced by BMPs in mesenchymal cells. Promoter analysis of Id1 indicates three distinct sequence elements that are sufficient and essential for efficient BMP-induced activation. Id1 was found to have an important effector function in various BMP-induced biological responses, including osteoblast differentiation.
The *SOST* gene product sclerostin is an osteocyte-derived negative regulator of bone formation and its deficiency causes the two closely related, rare skeletal dysplasia sclerosteosis and van Buchem disease. Sclerostin was found to inhibit BMP-stimulated bone formation *in vitro* and *in vivo*. Transcriptional profiling of osteoblastic cells treated with BMP in the absence or presence of sclerostin indicated that sclerostin specifically affects BMP and Wnt signaling among many other growth signaling pathways. Sclerostin, however, did not abrogate stimulation of direct BMP target genes, nor did we obtain any evidence for sclerostin acting as a direct BMP antagonist using a BMP specific reporter construct. In contrast, sclerostin shared many characteristics with the Wnt antagonist dickkopf-1 in antagonizing BMP-stimulated bone formation and BMP and Wnt-induced Wnt reporter construct activation. In conclusion, sclerostin abrogates BMP-stimulated bone formation by antagonizing Wnt signaling rather than directly inhibiting BMP signaling. High bone mass in sclerosteosis and van Buchem disease may, therefore, result from increased Wnt signaling.

S2.2

Integration of skeletal regulatory signals in nuclear microenvironments

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The architecturally associated subnuclear organization of nucleic acids and cognate regulatory factors suggests functional interrelationships between nuclear structure and gene expression. Mechanisms that contribute to the temporal/spatial distribution of transcription factors within the three dimensional context of nuclear architecture control the sorting and integration of regulatory information as well as the dynamic combinatorial assembly, organization and activities of transcriptional machinery at scaffold-associated subnuclear sites that support genetic and epigenetic control of gene expression. During the past several years, our laboratory has been addressing intranuclear trafficking mechanisms that direct transcription factors to transcriptionally active nuclear microenvironments. We are pursuing these studies using the AML/Runx/Cbfa transcription factors that govern hematopoietic and bone-specific transcription as a paradigm. Our objective is to gain insight into linkage of intranuclear organization of genes, transcripts, and regulatory proteins with fidelity of biological control and contributions of aberrant nuclear structure/function relationships to the onset and progression of tumorigenesis.
Our findings, from the combined application of molecular, cellular, biochemical and *in vivo* genetic approaches together with genomics and proteomics, demonstrate 1) a spatio-temporal mitotic partitioning and reorganization of regulatory factors that render progeny cells competent for the expression of RNA polymerase 1- and 2-mediated cell growth and tissue-specific genes that support cell fate and lineage commitment; 2) perturbations in subnuclear targeting of AML1 (Runx1) that are functionally coupled with competency for myeloid differentiation and expression of the transformed/leukemia phenotype; and 3) impaired intranuclear trafficking of AML3 (Runx2) transcription factors in metastatic breast cancer cells inhibits formation of osteolytic lesions in bone *in vivo*. Mechanisms that mediate the dynamic organization of gene regulatory machinery in nuclear microenvironments, which are compromised in cancer can provide a platform for novel approaches to diagnosis and therapy.

S2.3

TGF-β family ligands in glucose and fat homeostasis in adults

Alan Schneyer[1], Susan Bonner-Weir[2], Abir Mukherjee[4], Yisrael Sidis[3] & Melissa Thomas[3]
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The bioavailability of activin, GDF11, and myostatin, members of the TGFβ superfamily, is regulated by the soluble antagonists follistatin (FST) and follistatin like-3 (FSTL3). Activin influences tissue fate determination and organogenesis in embryos as well as organ homeostasis in adults while myostatin decreases muscle mass in adults and GDF11 regulates pancreatic β-cell differentiation. In addition, the FST gene produces three protein forms (FST288, FST303, and FST315) with different bioactivities and distributions within the body. Despite a great deal of biochemical and molecular knowledge, the precise physiological roles of FSTL3 and FST in regulating TGFβ ligands in the adult remain to be determined.
We have made several mouse genetic models designed to investigate the biological roles of FST and FSTL3 in adults. In contrast to FST KO mice, which die at birth, FSTL3 KO mice are born and survive like WT littermates. These mice, however, have larger testes, suggesting that FSTL3 regulates activin-mediated Sertoli cell proliferation. FSTL3 KO mice also have a number of metabolic phenotypes including enlarged pancreatic islets with β-cell hyperplasia, enhanced glucose tolerance and insulin sensitivity, hepatic steatosis, and reduced visceral fat mass. These enlarged islets are not evident in 2–3 months old mice indicating that activin and/or GDF11 have important roles in regulating islet size and β-cell number in adults. We have also made mice in which only the FST288 isoform is expressed (no circulating FST315 isoform). These FST288-only mice are viable and initially appear healthy, indicating that the FST288 isoform is sufficient to circumvent the global FST KO lethality. However, FST288-only mice exhibit reduced fertility. Moreover, they also develop enhanced glucose tolerance and insulin sensitivity, as well as hepatic steatosis, similar to the FSTL3 KO mice. Taken together, these mouse models indicate that FSTL3 and FST315, presumably through regulation of activin, GDF11 and myostatin bioactivity, have previously unappreciated activities in adults that may influence islet size and composition, glucose homeostasis, fat metabolism, and liver disease.

S2.4

The dependence receptor notion: when apoptosis regulates tumor progression and metastasis

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Dependence receptors are receptors that display two totally different signal transductions depending on ligand availability. If in the presence of ligand, these receptors transduce a positive signal leading to differentiation/proliferation/migration, in the absence of ligand these receptors induce an active process of cell death. Thus, such receptors create cellular states of dependence on their respective ligands by inducing apoptosis when unoccupied by ligand. This growing family of such bi-functional receptors now includes p75ntr, DCC, UNC5H1-3, neogenin, Patched, the androgen receptor, some integrins and two tyrosine kinase receptors RET and TrkC. As example, the DCC (Deleted in Colorectal Cancer), a candidate tumor suppressor, which encodes a receptor for netrin-1, a laminin-related molecule involved in axon guidance and UNC5H, another netrin-1 receptors have been shown to be pro-apoptotic unless netrin-1 is present. In the absence of netrin-1, DCC and UNC5H are cleaved by caspases and release/expose a pro-apoptotic domain that is able to drive apoptosis through interaction with pro-apoptotic proteins. Remarkably both DCC and UNC5H expression appears drastically inhibited in numerous carcinomas including colorectal tumors. We then have proposed that UNC5H and DCC may be considered as tumor suppressors. Indeed, ectopic expression of netrin-1 or inactivation of UNC5H3 in mice gut leads to i) a decreased cell death in the intestinal epithelium and ii) an increased tumorigenesis. Thus dependence receptors may turn as sensors that limit tumor growth out of ligand availability by inducing apoptosis. Here we will provide an overview of the implication of the netrin-1 dependence receptors in primary cancer and metastasis and will describe how this may be used to propose novel therapeutic approaches.

Gene/environment interactions in autoimmune endocrine disease – S3

S3.1

Why is the incidence of autoimmune diseases increasing in the modern world?

Jean-Francois Bach
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Western countries are being confronted with a disturbing increase in the incidence of most immune disorders, including autoimmune and allergic diseases. Converging epidemiological evidence indicates that this increase is linked to improvement of the socio-economic level of these countries. Epidemiological and clinical data support the hygiene hypothesis according to which the decrease of infections observed over the last three decades is the main cause of the incessant increase in immune disorders.

Independently of the need for confirmation by epidemiological prospective studies, the hygiene hypothesis still poses numerous questions concerning the nature of protective infectious agents, the timing of their involvement with regard to the natural history of immune diseases and, most importantly, the mechanisms of protection.
Three orders of mechanisms are being explored. Antigenic competition is the first hypothesis (immune responses against pathogens compete with autoimmune and allergic responses). Its discussion in the context of homeostatic regulation of lymphocyte pools has shed new light on this hypothesis. Infectious agents may also intervene through components, which are not recognized as antigens but bind to specific receptors on cells of the immune system. Major attention has recently been drawn to Toll receptors and TIM proteins present on Th cells, which may express the function of the virus receptor. Another hypothesis deals with immunoregulation. Infectious agents stimulate a large variety of regulatory T cells (Th2, CD25+, Tr1, NKT,…) whose effects extend to other specificities than those which triggered their differentiation (bystander suppression).

S3.2

Genome-wide association studies and hormone-dependent cancers
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The cataloguing of human genes and determination of common genetic variation in the human genome presents the major challenge of determining how inherited genetic variation affects our health. Epidemiologists are responsible for assessing the proportion of specific diseases associated with particular genotypes, and how these genotypes interact with environmental and lifestyle factors in disease causation. The advent of the capacity to perform Genome-Wide Association Studies has led to a rapid series of findings relating common inherited variation with risk of common cancers and other diseases and phenotypes. These associations should lead to new mechanistic insights, as well as having the potential to offer individuals cancer risk assessment. GWAS performed on breast cancer have led to the identification of common polymorphisms in *FGFR2* and several other genes being reproducibly related to risk of breast cancer. GWAS in prostate cancer have identified several variants in the 8q24 region, as well as MSMB and several other genes as related to risk of prostate cancer. Translation of these findings into public health and clinical practice is complex, and made more complex by the sheer number of new findings. The new technologies that permit genome-wide assessment of common genetic variation in research studies, also permit the determination of these genotypes in individual consumers at low cost per genotype. The responsible incorporation of these new technologies into medical practice poses unprecedented challenges to our conventional models of evaluation of risk assessment tools in the population and the clinic.

S3.3

Gene environment interactions in type 1 diabetes
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The incidence of type 1A diabetes has been doubling approximately every two decades in multiple developed countries. This implies important environmental determinants that are either increasing or decreasing and influencing overall incidence. Recent studies indicate that extreme genetic risk can be defined for major defined genetic subsets. For instance, siblings of a patient with type 1A diabetes who have the highest risk HLA alleles DR3/4-DQ2/8 and in addition have inherited identical by descent the same two HLA haplotypes as their proband sibling have a risk of islet autoimmunity by age 10 of 80%, and 60% for diabetes (Aly *et al. PNAS* 2006). The risk for siblings with DR3/4-DQ2/8 but sharing only a single haplotype identical by descent is 20%. For the general population combining HLA DR, DQ typing with exclusion of protective DP alleles can identify a risk as high as 20% (Baschal *et al. Diabetes* 2007). Such a high risk indicates that triggering environmental factors are likely to be ubiquitous. In animal models activation of the innate immune system by a virus such as KRV or by viral mimics such as poly-IC can trigger diabetes in genetically susceptible animals. Specifically poly-IC combined with what may be a primary autoantigen of diabetes of the NOD mouse, namely insulin peptide B: 9–23, induces insulitis in normal mouse strains. In addition to triggering, environmental factors may influence overall risk as illustrated by a recent report of omega-3-fatty acid decreasing risk of activating anti-islet autoimmunity in young children (Norris *et al. JAMA* 2007). A series of newly discovered genes associated with type 1 diabetes, apparently acting at the level of the immune system, will help define immunopathogenesis of type 1 diabetes, with fewer loci contributing to genetic prediction.

S3.4

Gene–environment interactions in thyroid autoimmunity
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The prevalence of overt autoimmune thyroid disease (AITD) is no less than around 2–3% in the adult population and increases with age. Subclinical thyroid autoimmunity is around 10 times higher, with a female preponderance. Although the major phenotypes (Graves' disease, Hashimoto's thyroiditis – with or without goitre, and chronic asymptomatic thyroiditis) are well characterized our knowledge of the aetiology of these disorders is incomplete. Studying twins constitutes an ideal starting point in the evaluation of a possible genetic background for a given disorder, and the evaluation of possible aetiological environmental factors is facilitated by the study of disease discordant twins. Despite this, very little research has been conducted using twins as a resource. Studies in twins suggest that there is a considerable genetic susceptibility in the aetiology of AITD, based on higher concordance rates (around 30–50%) in monozygotic (MZ) than in dizygotic (DZ) twins (around 5%). The main environmental factors seem to be related to iodine intake and cigarette smoking. Other suggested players (such as: low birth weight and various infections) are less likely.
The hurdle to overcome in the next years is to define, using e.g. molecular biological techniques, the genetic background for these phenotypes (so far with limited success), to disclose the relative contribution of environmental factors, alone or in concert, and to dig deeper into the understanding of gene–gene, environment–environment, and gene–environment interactions. The possibilities are of Galactic dimensions and the hitherto used methodology quite insufficient. The ultimate goal is a better understanding of the aetiology of AITD, enabling a more focused intervention and ultimately eradication of some phenotypes.

Is there a crisis for male reproduction – S4

S4.1

Deterioration of male reproductive function in Europe: geographical differences
Nils Joergensen

Abstract unavailable

S4.2

Life style factors and chemical exposures impair male reproductive development and function in humans
Katharina Main
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The prevalence of congenital malformations such as hypospadias and cryptorchidism in boys has increased over the past decades, following time trends and geographic distribution of adult reproductive disorders such as testicular cancer and impaired semen quality. This suggests a common prenatal origin of a testicular dysgenesis syndrome. During the first postnatal months, the pituitary–gonadal axis is activated, which can be used as a diagnostic window of testicular function close to suspected adverse effects *in utero*.
In our prospective, population-based cohort of 2562 newborn boys from Denmark and Finland, we found a higher prevalence of cryptorchidism and hypospadias in Danish than Finnish boys. Healthy Danish boys had smaller testes and showed less testicular growth during infancy, and their level of serum inhibin B was lower. In addition to low birth weight, being small for gestational age and prematurity, regular moderate maternal alcohol consumption and mild diabetes was found to be a risk factor for cryptorchidism. Environmental chemicals were measured in breast milk samples from mothers of cryptorchid and healthy boys. Positive associations were found between cryptorchidism and polybrominated diphenyl ethers (flame retardants) and polychlorinated pesticides. The content of phthalate monoesters (plastic emollients) was negatively correlated with serum testosterone levels.
Our findings suggest, that perinatal exposure to some environmental chemicals may have adverse effects on testicular development in humans. This is of concern, as the foetus and infant will be persistently and simultaneously exposed to many chemicals.

S4.3

Metabolic syndrome in Klinefelter syndrome (47,XXY)

Anders Bojesen
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Klinefelter syndrome (KS) is the most common sex chromosome disorder affecting 1:600 men.
We know from previous clinical and epidemiological studies that men suffering from KS have an increased risk of diabetes and an increased risk of dying from diabetes, but the reasons for the increased risk are unknown.
Seventy KS patients and 71 age-matched men participated in a study on body composition, measures of sex hormones, insulin sensitivity, blood pressure and various risk factors for ischemic cardiac disease.
Almost half of the KS patients fulfilled the NCEP/ATPIII criteria for the metabolic syndrome, whereas it was true for only 10% of the control group. Curiously, there was no difference in blood pressure between the two groups. Plasma lipids including LDL cholesterol were increased, while HDL cholesterol was decreased. Significantly more KS subjects had elevated fasting plasma insulin levels and calculation of insulin sensitivity using HOMA2 modelling showed a significant decrease in insulin sensitivity. KS subjects had excessive amounts of body fat and especially truncal fat. Multivariate analysis showed that the truncal obesity was the major determinant of both metabolic syndrome and decreased insulin sensitivity, even when controlled for testosterone and other androgens.
KS patients had normal values of adiponectin despite increased amount of body fat. This unexpected finding may in part explain the normal blood pressure, contrary to what would be expected from all the increased risk factors for ischemic cardiac disease. The potential cardioprotective effect of hypogonadism or the syndrome *per se* is reflected by a significant decreased mortality from ischemic cardiac disease found in a recent epidemiological study.

S4.4

Metabolic aspects of testosterone replacement

Michael Zitzmann
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Circumstances of life and food supply have changed in developed countries, resulting in an increasing prevalence of overweight. As a consequence, a complex disorder consisting of visceral obesity, dyslipidemia, insulin resistance and hypertension emerges: the so-called metabolic syndrome leads to the manifestation of diabetes type 2 and cardiovascular disease.
In men, testosterone deficiency contributes to the generation of the metabolic syndrome, as demonstrated by epidemiological and interventional approaches. Correspondingly, testosterone substitution in hypogonadal men is able to invalidate the mechanisms of the metabolic syndrome by various pathways. It has reciprocal effects on the generation of muscle and visceral fat tissue by exerting influence on the commitment of pluripotent stem cells. In addition, testosterone inhibits further development of pre-adipocytes. It also enhances insulin sensitivity of muscle cells by augmenting mitochondrial capacity and fostering expression of oxidative phosphorylation genes. These effects are exerted via androgen receptor-mediated mechanisms. Epidemiological and first interventional approaches indicate that testosterone substitution is especially helpful in preventing or attenuating disturbances of glucose metabolism and the metabolic syndrome in aging men with late-onset hypogonadism and in Klinefelter patients. However, large-scale double-blind placebo-controlled interventional studies of testosterone substitution therapy are required to corroborate these findings.

Protein modifications / proteomics – S5

S5.1

Comparative proteomics as a tool in biology: the protein microscope

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Department of Medical Genetics, University of Gothenburg, Gothenburg, Sweden

We use redundant peptide counting as a tool to compare, quantitatively, data from mass spectrometry analyses. When combined with hierarchical clustering, a tool commonly used for micro array analysis, we can group proteins based on their relative abundance across samples. In this way, we recently completed a spatial map of the secretory pathway of rat hepatic cells comprising some 1400 different proteins, each with a defined distribution (Gilchrist, Au, Hiding *et al*. 2006 *Cell* **127** 1265–81). Using the same approach, we have now analyzed the secretome of mouse embryonic fibroblasts that were differentiated, in vitro, into adipocytes. By comparing the secretome of such adipocytes taken from mouse knock-in or knock outs of FOXC2, a transcription factor that when over expressed, shifts white adipose tissue towards brown adipose tissue (Cederberg *et al*. *Cell* **106** 563–73), we have identified several secreted factors that are distinct for each mouse strain. Their relevance to white and brown adipose tissue will be discussed.

S5.2

Applying proteomics to thyroid tumour diagnosis

Dagmar Fuhrer
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The application of proteomics in clinical thyroid logy is innovative and is a novel tool to decipher Pathophysiologie-inherent changes in protein function, which cannot be comprehensively studied by the more widely applied transcriptome methods.
In this talk we will provide an overview on methods of thyroid proteomics and their possible pitfalls, present current studies on this topic and discuss our own experience with the application of proteomics technology to study differential protein expression in benign cold thyroid nodules (CTN).
Thus, using a combined approach of 2D gel-electrophoresis (2D-GE) and mass spectrometry, followed by western blot analysis and immunohistochemical studies, we demonstrate for the first time, that benign CTNs are characterised by the up-regulation of several components of the thyroid hormone synthesis machinery, with a strong induction of the H_2O_2 generating and detoxifying systems. Moreover, increased formation of 8-*oxo*-guanidine DNA-adducts was detected in CTNs and could besides the increased cell proliferation in these nodules confer an increased potential for genotoxicity and mutagenesis.

S5.3

Targeting ubiquitin networks

Ivan Dikic
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Ubiquitin (Ub) plays a critical role in many fundamental processes like cell cycle, apoptosis, DNA repair or endocytosis. In these processes Ub acts as a signalling component able to trigger molecular events in cells. Ub does so by operating as a reversible and highly versatile regulatory signal for an expanding number of Ub-binding domains (UBD) present in cellular proteins that convey Ub signals into appropriate cellular phenotypes. In addition, it is becoming apparent that deregulation of Ub pathways results in the development of human diseases including many types of tumours. The identification and structural characterization of novel Ub binding domains (UBZ and Pru domains), and their functional involvement in the regulation of NFκB pathway and proteasomal functions will be presented.

S5.4

Progesterone receptor responses modified by SUMO in the endometrium

Jan Brosens
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Cyclic AMP sensitizes human endometrial stromal cells (HESCs) to progesterone and promotes differentiation into decidual cells, a process indispensable for pregnancy. Our studies have shown that cAMP enhances progesterone responses, at least in part, by attenuating ligand-dependent sumoylation of the progesterone receptor (PR). In fact, decidualization is associated with global hyposumoylation and redistribution of SUMO-1 conjugates into distinct nuclear foci. This altered pattern of global sumoylation is not attributable to impaired maturation of SUMO-1 precursor or altered expression of E1 (SAE1/SEA2) or E2 (Ubc9) enzymes but coincides with profound changes in the expression of E3 ligases and SUMO-specific proteases. Specifically, concomitant down-regulation of PIAS1 and up-regulation of the de-conjugating isopeptidase SENP2 limit SUMO-1 modification of ligand-bound PR in decidualizing cells, thereby enhancing the ability of the receptor to activate reporter constructs and endogenous genes. The activity of SUMO conjugating and de-conjugating enzymes is regulated by a

variety of environmental stress signals, including free radicals, suggesting that sumoylation serves as a major mechanism for sensing and responding to environmental changes. Interestingly, free radicals such as hydrogen peroxide strongly activate JNK and ERK1/2 signalling, perturbs the cellular sumoylation–desumoylation equilibrium, and impairs progesterone responses in undifferentiated HESCs. In decidual cells, however, selective silencing of JNK activation uncouples the SUMO pathway from oxidative stress signals, thereby ensuring that progesterone responses and cellular homeostasis are maintained.

Insights in pancreatic development and new clinical aspects – S6

S6.1

Embryonic pancreatic development

Francesca M Spagnoli
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The formation of the vertebrate pancreas is a complex process that typifies the basic steps of embryonic development. It involves the establishment of competence, specification, signaling from neighboring tissues, morphogenesis, and the elaboration of tissue-specific genetic networks. A full analysis of this multistep process will help us to understand classic principles of embryonic development. Furthermore, this will provide the blueprint for experimental programming of pancreas formation from embryonic stem cells in the context of diabetes cell-therapy. Although in the past decade many studies have contributed to a solid foundation for understanding pancreatogenesis, important gaps persist in our knowledge of early pancreas formation. This is particularly true for stages between endoderm formation and initiation of organogenesis.

Combining embryology and genomics in *Xenopus laevis*, we have characterized these early stages and identified: 1) a suite of early endodermal factors that establish a pancreatic pattern within the pluripotent endoderm, including the transcriptional modulator, TGIF2; and 2) an instructive signal sufficient to induce pancreatic differentiation and, notably, insulin expression in the embryo, such as the signaling factor, Shirin/DLC2. Subsequently, using a comparative developmental approach, we have showed that the biological activity of these novel inductive pancreatic signals is conserved in mammalian systems.

These findings implicate new and unexplored pathways in the early stages of pancreas formation. Further investigation of the biological function of these factors and integration into the network of previously defined determinants will elucidate the early mechanisms coming into play to pattern the pre-pancreatic region within the endoderm and, gradually, specify the pancreatic tissue.

S6.2

The zinc-finger factor Insm1 (IA-1) is essential for the development of endocrine cells of the pancreas, intestine and adrenal gland

Carmen Birchmeier, Mathias Gierl, Hendrik Wildner & Hagen Wende
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Endocrine cells of the intestine, pancreas and adrenal gland develop from the endoderm and neural crest, respectively. Despite their different developmental origins, we discovered that these distinct endocrine cells rely on a common transcription factor, Insm1, that controls their development. The Insm1 (insulinoma-associated 1, IA-1) gene encodes a zinc-finger factor that was discovered in an insulinoma cDNA library. We show that pancreatic and intestinal endocrine cells as well as the endocrine cells of the adrenal gland express Insm1 and require Insm1 for their development. In the pancreas of Insm1 mutant mice, endocrine precursors are formed, but only few insulin-positive beta cells are generated. Instead, endocrine precursor cells accumulate that express none of the pancreatic hormones. A similar change is observed in the development of intestine and adrenal gland, where endocrine precursor cells are generated but do not differentiate correctly. A hallmark of endocrine cell differentiation is the accumulation of proteins that participate in secretion and vesicle transport, and we find many of the corresponding genes to be down-regulated in Insm1 mutant mice. Insm1 thus controls a gene expression program that comprises hormones and proteins of the secretory machinery. Our genetic analysis has revealed a key role of Insm1 in differentiation of endocrine cells.

S6.3

Hyperinsulinism in humans

Khalid Hussain[1,2]
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Hyperinsulinism causes recurrent and severe hypoglycaemia in the newborn, infancy and childhood period. Although the condition is more common in the newborn period it can present even adults can present with late onset hyperinsulinaemic hypoglycaemia not due to an insulinoma (noninsulinoma pancreatogenous hypoglycemia). Hyperinsulinaemic hypoglycaemia encompasses a heterogeneous group of disorders with respect to clinical presentation, pancreatic histology and molecular biology. The commonest genetic cause of persistent hyperinsulinism are autosomal recessive mutations in the genes *ABCC8* and *KCNJ11* (encoding the two subunits SUR1 and KIR6.2 respectively) of the pancreatic ATP-sensitive potassium channel (K_{ATP}). Defects in these genes cause the most severe forms of hyperinsulinaemic hypoglycaemia which in some cases may require either a limited pancreatectomy (focal form of the disease which will 'cure' the patient) or a near total pancreatectomy (diffuse form with a high risk of diabetes mellitus). Recent advances in imaging techniques such as ^{18}Fluoro-L-Dopa PET (positron emission tomography) have completely changed the management of patients with severe form of hyperinsulinaemic hypoglycaemia as it allows accurately differentiation of focal from diffuse disease. Other rare genetic causes of hyperinsulinaemic hypoglycaemia include mutations in the *GCK* (glucokinase), *GLUD1* (glutamate dehydrogenase), *HAHD* (SCHAD), *HNF4A* (hepatocyte nuclear factor 4 alpha), *INSR* (insulin receptor) and *SLC16A1* (monocarboxylate transporter 1) genes. Hyperinsulinaemic hypoglycaemia may also be part of an underlying syndrome (such as Beckwith-Wiedemann, Costello and Kabuki) and multisystem disorders such as congenital disorders of glycosylation (CDG). Hyperinsulinaemic hypoglycemia following gastric bypass surgery for obesity has been reported in several adult patients with pancreatic histological changes similar to infants with persistent hyperinsulinism. The molecular mechanisms leading to the unregulated insulin secretion in these patients are unclear but glucagon like peptide 1(GLP-1) may have a role. During this talk I will give an overview of the different mechanisms leading to hyperinsulinaemic hypoglycaemia in humans.

S6.4

Carboxylester lipase (*CEL*) VNTR links exocrine dysfunction to endocrine dysfunction

Pål Njølstad
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The localization of the islets of Langerhans within exocrine pancreatic tissue is suggestive of an interdependency and cross-talk between these two cell populations, in their normal as well as in their abnormal function. Disturbances in glucose metabolism often accompany diseases of the exocrine pancreas, whereas exocrine dysfunction is commonly seen in diabetes patients. Recently, we have described a novel syndrome of exocrine and endocrine pancreatic dysfunction caused by mutations in the carboxyl-ester lipase (*CEL*) gene (Ræder *et al*. *Nat Genet* 2006). *CEL* is mainly expressed in the pancreatic acinar tissue, and the protein is secreted from the pancreas into the digestive tract. *CEL* is highly polymorphic due to a VNTR in exon 11. We have described two families with different, single-base deletions in this VNTR. Screening *CEL* in MODYX materials from the UK and Denmark indicates that *CEL* mutations may remain a rare cause of monogenic diabetes. Moreover, we have not found any association between VNTR status and type 2 diabetes.

MRI and ultrasound studies of non-diabetic and mutation-positive children suggest that mutation carriers accumulate fat in their pancreata. Moreover, this process seems to occur before the anticipated development of diabetes (Ræder *et al*. *Diabetes* 2007). In a treatment trial, nine affected subjects were given pancreatic enzyme substitution therapy. This improved their vitamin E status and increased their blood cholesterol level.

To examine the role of *CEL* in diabetes development, we have studied *CEL* knock-out mice. A mild diabetic phenotype was noted in female mice. Moreover, we have made epitope-tagged constructs of the mutated and wild-type *CEL* genes, expressed these constructs in cell lines and examined protein secretion and subcellular distribution. Preliminary data will be presented.

One possible mechanism of disease could be that lack of O-glycosylation of the mutant *CEL* causes retention and accumulation of the protein in the ER/Golgi apparatus of the acinar cells, leading to ER stress. Our data may support such a disease model, although impaired secretion or increased degradation of the protein are alternative explanations. Taken together, the molecular and clinical data are consistent with the syndrome starting in the acinar tissue and diabetes being secondary to the exocrine defect.

The functions of peripheral ghrelin – S7

S7.1

Ghrelin and the gonads

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Gonadal function is primarily driven by the tropic actions of pituitary gonadotropins (LH and FSH), whose secretion is in turn regulated by the hypothalamic decapeptide GnRH. This neuro-hormonal axis (namely, the HPG axis) is under the modulation of a wide diversity of regulatory signals, which include metabolic cues and factors controlling energy homeostasis. Among those, a dominant role of leptin, the adipose hormone signaling energy sufficiency, in the control of gonadal function has been substantiated in the last decade; leptin being a major effector for the metabolic regulation of fertility, at different levels of the HPG axis. Recent experimental data suggest that the gut hormone ghrelin, as signal of energy insufficiency and functional antagonist of leptin, may operate also as pleiotropic modulator of gonadal function and the reproductive axis. Such regulatory actions appear to be multi-faceted, as i) ghrelin has been shown to inhibit luteinizing hormone (LH) secretion in rodents and other mammals (including humans); ii) ghrelin operates a negative modifier of puberty onset in (male) rats; iii) ghrelin and its canonical receptor (the GHS-R1a) are expressed (and regulated) in the gonads; and iv) ghrelin is able to conduct direct gonadal actions; testicular effects of ghrelin include inhibition of testosterone secretion, Leydig cell proliferation and expression of relevant Sertoli cell genes. Thus, through direct and indirect actions, ghrelin has recently emerged as putative modulator of gonadal function, with predominant inhibitory effects in line with its proposed role as signal of energy insufficiency. Overall, it is tempting to propose that, in addition to 'classical' metabolic regulators (such as leptin), the gut hormone, ghrelin, is a 'novel' player in the dynamic regulation of gonadal function, which might contribute to the integration of energy balance and reproduction.

S7.2

Ghrelin and the endocrine pancreas

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Ghrelin was isolated from the stomach and identified as an endogenous ligand of the growth hormone secretagogue receptor type 1a (GHS-R1a). Numerous studies have demonstrated that acylated ghrelin, besides its main endocrine actions such as stimulation of GH secretion and regulation of energy balance, has a wide spectrum of peripheral activities which engage metabolic, endocrine, cardiovascular, bone and immune systems. It is now clear that also unacylated ghrelin, although unable to bind to the GHS-R1a, is a biologically active peptide, with functions mediated through an unknown receptor. In the endocrine pancreas, ghrelin has been shown to localize to α- and β-cells and to the newly identified ghrelin-producing islet ε-cells, suggesting a role in the regulation of β-cell fate and function. Accordingly, ghrelin was recently shown to prevent diabetes in streptozotocin-treated rats, by increasing β-cell mass and insulin secretion. Survival of β-cells is of major importance for maintaining normal glucose metabolism and β-cell apoptosis is a critical event in both type 1 and 2 diabetes. We recently demonstrated that both acylated and unacylated ghrelin promote proliferation and inhibit apoptosis of β-cells and human pancreatic islets through cAMP/PKA, ERK1/2- and PI3K/Akt-mediated mechanisms. Furthermore, both peptides stimulated glucose-induced insulin secretion by β-cells. Besides ghrelin, the ghrelin gene encodes for obestatin, whose biological functions are still largely unknown. Recently, we showed that obestatin, like ghrelin, promotes proliferation and survival of β-cell lines and human pancreatic islets through mechanisms involving the ghrelin system and the glucagon-like peptide-1 receptor (GLP-1R) signaling. Indeed obestatin, similarly to GLP-1R agonists, induced insulin secretion and expression in human islets and up-regulated the mRNA of genes that are main regulators of β-cell function, survival and differentiation. Therefore, the products of the ghrelin genes, ghrelin and obestatin, appear to play a key functional and survival role within the endocrine pancreas.

S7.3

Ghrelin and bone

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Gastrectomy causes rapid development of osteopenia that, in animal models at least, is independent of nutritional effects. Ghrelin is produced primarily in the stomach, suggesting a link between the loss of ghrelin and gastrectomy induced osteopenia. Ghrelin stimulates growth hormone (GH) secretion and could positively affect bone metabolism via the GH-IGF axis, although ghrelin receptor (GHS-R) deleted mice have unaltered BMD and BMC, despite having suppressed IGF-I serum levels. However, there is evidence that the GHS-R and ghrelin are expressed in bone cells *in vitro* as well as *in vivo*, and that osteoblasts respond to ghrelin through altered growth rate and pattern of differentiation, pointing to possible subtle effects on structural characteristics of bone. Moreover, in GH-deficient rats BMD is increased by infusion of ghrelin, demonstrating a GH-independent effect. Unacylated ghrelin (UAG), which activates the GHS-R only at supraphysiological concentrations, also appears to alter parameters of osteoblast growth, presumably via an alternate receptor. In partial corroboration of these findings, overexpression of UAG in mice leads to development of a small phenotype, contributed partly by reduced skeletal length. The clinical utility of ghrelin or activation of the GHS-R in bone disease is less clear, perhaps because of the paucity of studies. MK-677, a GHS-R agonist, has been demonstrated to synergise with bisphosphonate treatment in improving femoral head BMD, but not at other sites. Additionally, in a study of an older population, ghrelin shows an inverse association with NTx, a marker of bone turnover, in men but not women. In conclusion, recent evidence suggests that ghrelin and its unacylated isoform modulate bone growth in rodents independently of GH, and alter growth and differentiation of bone cells *in vitro*. Although clinical studies are limited, there is some evidence that ghrelin, or GHS-R agonists, can affect bone health in humans.

S7.4

Peptidyl growth hormone secretagogues in cardioprotection

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Peptidyl growth hormone secretagogues (GHS), including ghrelin and growth hormone releasing peptides (GHRPs), have been reported to exert cardioprotective effects in experimental models of ischemia-reperfusion (I/R) injury through GH-independent pathway. Along this line, we have identified CD36 as a main target for GHRP binding and action in the heart. Yet, not much is known regarding the molecular mechanisms of the cardioprotective effects of GHRPs. The objective of this study is to dissect the relative role of GHS-R1a and CD36 signaling in cardioprotection using both pharmacologic and genetic approaches. The effects of the selective CD36 ligand (EP 80317) and of hexarelin, a dual CD36 and GHS-R1a agonist, were assessed in murine myocardial I/R. Pretreatment of wild-type (WT) C57BL/6 mice for 2 weeks with either EP 80317 (300 μg/kg) reduced the infarct size in isolated hearts by 75% ($P<0.05$), following *ex vivo* global ischemia (30 min)/reperfusion (90 min). In contrast, no effect of the peptides were observed in CD36-null mice, suggesting that CD36 may play a cardioprotective role against I/R-elicited cardiac injury. One of the mechanism by which GHS may contribute to reduce myocardial I/R is by preventing early cardiomyocyte loss through apoptosis. In the present study, we investigated the effect of EP 80317 (300 μg/kg) and of hexarelin (100 μg/kg) on downstream effectors of the signaling pathways of PI3K/Akt in a model of transient (30 min) left coronary artery ligation in isoflurane-anaesthetized WT mice. After 6-h of reperfusion, Akt phosphorylation increased by 44% ($P<0.001$) and 8% (NS) in EP 80317- and hexarelin-treated mice, respectively. Phosphorylation of Akt was not enhanced in treated CD36-null mice. These results support a critical role for CD36 in mediating GHRP cardioprotective effect, at least in part through the PI3K/Akt signaling mechanisms leading to the phosphorylation and activation of Akt.

Nuclear receptors – S8

S8.1

LXR

E Saez

Abstract unavailable

S8.2

Kinase signaling and retinoic acid nuclear receptors

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It is well established that nuclear retinoic acid (RA) receptors (RARα, RARβ and RARγ) activate gene expression through a complex and evergrowing network of dynamic interactions with coregulatory protein complexes, which are coordinated by the ligand.
Adding more complexity to this scenario, we demonstrated that RARs are also targets for phosphorylation processes, which govern their transcriptional activity in cooperation with the ligand. Indeed, following RA addition, p38MAPK is rapidly activated, resulting in the phosphorylation of the C-terminal Ligand-Binding Domain of RARs. This phosphorylation process controls the recruitment of the cdk7/cyclin H/MAT-1 subcomplex of the general transcription factor TFIIH through the docking of cyclin H and therefore the phosphorylation of the N-terminal domain of RARs by cdk7. The RAR coregulators (SRC-3/AIB1) are also phosphorylated by the p38MAPK pathway. These RA-induced phosphorylation processes control the dynamics of the interactions between RARs and their coregulators. Their also control the recruitment of RARs at response elements within the promoters of target genes. Then phosphorylation signals the end of transcription via promoting the degradation of RARs and their coactivators.
We propose a model in which RAR activity is regulated by a phosphorylation code defined by two kinases, cdk7/TFIIH and p38MAPK. This code participates in cooperation with the ligand to switch on and off the RA response of RAR-target genes. This would be an intervention point for drug development for cancers caracterized by aberrant kinase activities.

S8.3

Phosphorylation of histone H3 at threonine 11 establishes a novel chromatin mark for transcriptional regulation

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Post-translational modifications of histones such as methylation, acetylation, and phoshorylation regulate chromatin structure and gene expression. However, phosphorylation of histone H3 at threonine 11 (H3T11ph) has not been linked to transcriptional regulation. Here we show that protein kinase C-related kinase 1 (PRK1) phosphorylates H3T11 upon ligand-dependent recruitment to androgen receptor (AR) target genes. PRK1 is pivotal to AR function since PRK1 knockdown by RNAi or PRK1 inhibition by treatment with Ro318220 impedes AR-dependent gene expression. Blocking PRK1 function abrogates androgen-induced H3T11 phosphorylation, and in consequence, inhibits androgen-induced demethylation of histone H3 *in vivo*. Moreover, the presence of serine 5-phosphorylated RNA polymerase II is no longer observed at AR target promoters. *In vitro*, phosphorylation of H3T11 by PRK1 accelerates demethylation by the Jumonji C (JmjC) domain-containing protein JMJD2C[3]. Thus, -phosphorylation of H3T11 by PRK1 establishes a novel chromatin mark for transcriptional activation, identifying PRK1 as a gatekeeper of AR-regulated gene expression. Importantly, elevated PRK1- and H3T11ph levels positively correlate with high Gleason scores of prostate carcinomas, and inhibition of PRK1 blocks AR-induced tumour cell proliferation. Thus, PRK1 is a promising therapeutic target in the treatment of prostate cancer.

S8.4

Estrogen-related receptors as regulators of energy homeostasis and adaptive metabolism

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Adaptation of oxidative metabolism is essential for meeting different energy demands in different tissues, or altered energy demands in response to stressors, such as exposure to cold. The orphan nuclear receptor ERRα (estrogen-related receptor alpha) is implicated in the regulation of energy metabolism. ERRα expression is high in tissues with high oxidative capacity and increased at physiologic states of increased energy demand. Moreover, ERRα activity and expression are regulated by the co-activators PGC-1α and PGC-1β, which integrate signals indicating changing energy demands. Gain- and loss-of function studies *in vitro* show that ERRα is a crucial downstream effector of PGC-1α and PGC-1β in the regulation of several facets of mitochondrial metabolism, including mitochondrial biogenesis and oxidative capacity. To address the physiologic function of ERRα *in vivo* we have studied mice lacking ERRα and shown that they are defective in adaptive thermogenesis. The impaired thermogenic function is not due to defects in the acute transcriptional induction of 'thermogenic' genes, suggesting that ERRα is dispensable for some subsets of PGC-1α-controlled programs. Rather, ERRα is needed for the high levels of mitochondrial biogenesis and oxidative capacity characteristic of brown adipose tissue, and thus for providing the energy necessary for thermogenesis. ERRα fulfils this role by binding directly and enhancing the expression of genes important for fatty acid oxidation, TCA cycle, and oxidative phosphorylation, acting parallel to other transcription factors that control mitochondrial gene expression, such as NRF1 and NRF2/GABPA. These findings demonstrate that ERRα is an important component of the network that controls adaptive mitochondrial biogenesis and function *in vivo*, and essential for survival in situations of high energy demand. The physiologic consequences of ERRα lack of function in other tissues and the extent to which other members of the ERR family compensate for the lack of ERRα will be discussed.

New aspects of adrenal disease – S9

S9.1

The role of toll-like receptors

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The first characterised mammalian Toll-like receptor (TLR) was described in 1997, i.e. TLR4, which can detect lipopolysaccharide (LPS) from Gram-negative bacteria. Since then several proteins structurally related to TLR4 were identified (TLR1-10). For example, TLR2 can bind to lipopeptides from Gram-positive bacteria. For both receptors human polymorphisms have been identified (TLR4 up to 14% and TLR2 up to 10% in Europe) and linked to several clinical conditions such as asthma, coronary artery stenosis, myocardial infarction, peridontitis, renal transplantation, stroke or ulcerative colitis as well as burn trauma and *S. aureus* infection – just to name a few. Numbers of patients enrolled in these polymorphism studies vary between 20 and 2000. At present, these studies demonstrate controversial results in terms of outcome. This might be explained by the fact that only small patient numbers have been used as well as that some of these trials did not study patient outcome as an endpoint. Recent data from our lab demonstrate a novel link between the innate immune system and the endocrine stress response mediated by TLRs. TLR2 and TLR4 are expressed in human and mice adrenals and TLR2 deficiency is associated with an impaired glucocorticoid response. We present here pre-clinical data and results of a trial (Toll Police S1 = *Toll*-like receptor *Pol*ymorphism *i*n *c*ardiovascular *e*lective *S*urgery Trial *1*) conducted in 450 patients undergoing elective cardiac surgery. TLR2 and TLR4 polymorphisms were determined and correlated with 28 days mortality (among other factors) following surgery. The lecture will present pre-clinical results and design, population and outcome data from the trial. Furthermore, we will highlight limitations and drawbacks as well as suggestions for future trials.
Reference
Bornstein *et al*. *Horm Metab Res* 2004; Bornstein *et al*. *PNAS* 2004; Zacharowski *et al*. *PNAS* 2006; Tran *et al*. *J Clin Endo Metab* 2007; Kanczkowski *et al*. *Horm Metab Res* 2007.

S9.2

The expanding spectrum of DAX1 and SF1 mutations

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DAX1 (NR0B1) and steroidogenic factor-1 (SF1, NR5A1) are two nuclear receptors that play a central role in adrenal development and disease. DAX1 was discovered as the cause of X-linked adrenal hypoplasia congenita in 1994 and, to

date, more than 250 individuals and families with this condition have been reported. Boys tend to present with salt-losing adrenal failure in the neonatal period or with signs and symptoms of glucocorticoid insufficiency throughout childhood. Hypogonadotropic hypogonadism or pubertal arrest occurs at adolescence and impaired spermatogenesis is usually seen. More recently, the phenotypic spectrum associated with DAX1 mutations has been expanded to include apparent isolated mineralocorticoid deficiency, premature sexual maturation in childhood, or delayed-onset milder adrenal failure and partial hypogonadotropic hypogonadism first presenting in adulthood. Furthermore, female heterozygous carriers of DAX1 mutations may show adrenal failure or pubertal delay due to skewed X-inactivation. Thus, DAX1 mutations are a common cause of adrenal hypoplasia in boys, and an almost invariable cause when a family history of X-linked adrenal disease and hypogonadism are present. In contrast, although SF1 is a key regulator of adrenal and development and function in *in vivo* and *in vitro* studies, SF1 mutations are not a common cause of severe adrenal dysfunction in humans. To date, three patients with primary adrenal failure (two 46,XY females; one 46,XX female) due to SF1 mutations have been reported. However, haploinsufficiency of SF1 is emerging as a relatively frequent cause of 46,XY DSD (variable testicular dysgenesis and impaired androgenization) or vanishing testis syndrome, and polymorphisms in SF1 have been associated with micropenis or undescended testes. Long-term follow up of adrenal function will be needed in these individuals. Furthermore, somatic copy number changes resulting in overexpression in SF1 are reported in a proportion of paediatric adrenal tumors. Taken together, DAX1 and SF1 play important and diverse roles in human adrenal and reproductive function and variations in these genes can present with a spectrum of endocrine disorders in adulthood as well as in childhood.

S9.3

Replacement and QoL In patients with adrenal insufficiency

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Adrenal insufficiency is a deadly disorder. Studies on the outcome of patients with adrenal insufficiency may have been hampered by the dramatic improvement in mortality and morbidity that occurred when synthetic glucocorticoids came available more than 60 years ago. Studies during the last 17 years have repeatedly shown that mortality rate in patients with hypopituitarism is doubled. The presence of adrenal insufficiency in hypopituitarism in young adults with childhood-onset disease has been associated with increased mortality rate, but not in adults with adult-onset disease. In patients with primary adrenal insufficiency survival rate has been considered to be normal. Recent data, however, suggest that these patients may have excess mortality due to cardiovascular-, infectious diseases and malignancy. Studies on morbidity in patients with primary adrenal insufficiency are few with some indicating impaired glucose tolerance, reduced bone mineral density and impaired QoL and well-being. Current strategy for glucocorticoid replacement therapy is based on 2–3 administrations of short-acting hydrocortisone or the administration of a more long acting synthetic glucocorticoid. Historically overly high daily doses of glucocorticoids for replacement may have caused metabolic and catabolic side-effects. The increased mortality due to infectious disorders also suggests inadequate replacement therapy under such circumstances. The impact of an unphysiological diurnal exposure of glucocorticoids with current replacement strategy is less clear. Producing a more physiological serum cortisol curve have in some studies improved well being either by increasing the frequency of oral hydrocortisone administration or by using a continuous infusion of hydrocortisone. Recent attempts have also been made to include hydrocortisone into novel drug delivery techniques making the diurnal exposure of cortisol more physiological. Also, delivery systems allowing once-daily administration simplify treatment and should thereby improve compliance with possible beneficial effects on short- and long-term outcome in adrenal insufficiency.

S9.4

Genetics of chromaffin tumours

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Recently, clinical and fundamental research studies have dramatically changed the knowledge on the genetics of pheochromocytoma (PH). Previously, it was widely accepted that only 10% of the patients affected by a PH had a familial disease and that the malignant phenotype of a PH could not be diagnosed before the occurrence of the first metastasis. After the identification of the genes involved in the hereditary paraganglioma/pheochromocytoma syndrome (*SDHD*, SDHB, *SDHC*)[1–3], it has been demonstrated that 25% to 30% of the patients have a hereditary PH due to a germline mutation on SDHB, SDHD, VHL, *RET* or *NF1* genes[3–4] and that the identification of a *SDHB* mutation is a high risk factor for malignancy and poor prognosis[4–6]. Those data have supported new recommendations for genetic counselling and genetic testing as well as for the management of the affected patients[7–9]. Moreover, fundamental research studies have contributed to the understanding of new molecular mechanisms involved in the PH tumorigenesis. In particular, it has been shown that *SDHs* genes are new mitochondrial tumour suppressive genes and that the succinate dehydrogenase inactivation induces an abnormal stimulation of the hypoxia-angiogenesis pathway[10–11].

1. Baysal BE. *J Med Genet* 2002 **39** 617–622.
2. Favier J *et al*. *Horm Res* 2005 **63** 171–9.
3. Neumann HP *et al*. *N Engl J Med* 2002 **346** 1459–66.
4. Amar L *et al*. *J Clin Oncol* 2005 **23** 8812–8.
5. Gimenez-Roqueplo AP *et al*. *Cancer Res* 2003 **63** 5615–21.
6. Amar L *et al*. *J Clin Endocrinol Metab* 2007 **92** (10) 3822–8.
7. Plouin PF & Gimenez-Roqueplo AP. *Nat Clin Pract Endocrinol Metab* 2006 **2** 60–61.
8. Gimenez-Roqueplo AP *et al*. *Clin Endocrinol* 2006 **65** 699–705.
9. Pacak *et al*. *Nat Clin Pract Endocrinol Metab* 2007 **3** 92–102.
10. Gimenez-Roqueplo AP *et al*. *Am J Hum Genet* 2001 **69** 1186–1197.
11. Gimenez-Roqueplo AP. *Ann N Y Acad Sci* 2006 **1073** 112–21.

Pro & Con – is there a rationale for ISS treatment with GH – S10

Abstracts Not Required

Epigenetics in endocrinology – S11

S11.1

Epimutations in the human genome

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Gene expression states are set by transcriptional activators and repressors and often locked in by cell-heritable chromatin states. Aberrant chromatin states leading to aberrant gene expression patterns (epimutations) can change developmental trajectories and result in disease. Epimutations have been detected in several recognizable syndromes, especially those involving imprinted genes, as well as in cancer. They can result from a DNA mutation in a cis- or trans-acting epigenetic factor (secondary epimutation), or occur as a 'true' or primary epimutation in the absence of any DNA sequence change. Primary epimutations often occur after fertilization and lead to somatic mosaicism. It has been estimated that the rate of primary epimutations is one or two orders of magnitude greater than somatic DNA mutation. Therefore the contribution of epimutations to human disease is probably underestimated.

S11.2

Mechanisms of selective gene expression in development

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The concept of tissue-specific gene expression is familiar to endocrinologists. Starting from the multipotent fertilized egg, selective gene expression is required to generate different tissue and cell types during development. There is generally progressive differentiation of initially dividing, but progressively committed, cells to form mature organs. Timing, size and positioning of organs and the setting up of connections between them all require complex regulation by networks of transcription factors and signaling molecules, which are also involved in

controlling the final stages of differentiation. Regulated gene expression is also necessary for continuing finely-tuned differentiated and adult function, for example controlled endocrine activity. Gene expression is regulated at several different levels, some of which are inter-dependent. Firstly, DNA itself can be modified, for example by methylation. Chromatin organization – the compacting of DNA – is effected through differential binding of general and specific proteins. Accessibility of DNA for transcription is controlled, at one level, by secondary modifications (e.g. methylation, acetylation) of chromatin proteins including histones and structural non-histone molecules. Gene expression is modulated by the binding of upstream transcription factors, which may act as repressors or as activators. Often several TFs work together in concert, frequently showing complex auto- and cross-regulation. Their functions are highly dosage sensitive and their exact expression is mediated through the binding of hierarchies of multiple TFs to complex conserved interactive DNA sites, often lying long distances from the transcribed gene. Many developmental anomalies, for example of the pituitary or the pancreas, are caused by TF mutations.

S11.3

Epigenetics in mammalian development

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The classical way of thinking about the development of the early mouse embryo has been that it is an essential prerequisite for cells to adopt differential, inside or outside positions for them to acquire differential expression of cell fate determining genes. However, over recent years it has been realised that important differences arise between cells in gene expression, cell fate and potential earlier than previously realised, before cells acquire specific positions. We have found that levels of methylation of histone H3 at specific arginine residues in 4-cell blastomeres correlate with developmental potential in a way that suggested that this epigenetic modification might predispose blastomeres to contribute to the pluripotent cells of the ICM. This prediction was confirmed by the finding that when the H3-specific arginine methyltransferase, CARM1, was overexpressed in individual blastomeres, it directed their progeny to contribute to the ICM. This suggests that arginine methylation of H3 contributes to development of the ICM and identifies this modification as the earliest known epigenetic marker in the process.

S11.4

Imprinting in human disease: lessons from the study of transient neonatal diabetes

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Imprinted genes differ from most gene pairs in that only one gene of the pair is expressed determined by the parent they originate from. Many have been shown to play an important role in fetal growth and neurodevelopment. Most imprinted genes are found in clusters and expression is controlled by imprinting centres that contain differentially methylated regions. Monoallelic expression makes imprinted genes particularly vulnerable to naturally occurring genetic rearrangements and epigenetic alterations. At least eight disorders known to be due to imprinting errors are now well recognised; Prader Willi syndrome (15q11–13), Angelman syndrome (15q11–13), Transient Neonatal Diabetes (6q24), Beckwith Wiedemann syndrome (11p15.5), Silver Russell syndrome (11p15.5 and UPD7 -locus not known), Maternal and Paternal UPD 14 (14q32) and Pseudohypoparathyroidism Type 1B (20q13). There are some overlapping clinical features particularly aberrant growth, abnormal glycaemic control and developmental delay.
Transient neonatal diabetes (TND) is due to imprinting aberrations at 6q24 involving ZAC over-expression. Patients present in the neonatal period with diabetes and low birth weight and require insulin for an average period of 3 months. Non diabetic features such as macroglossia and umbilical hernia have been underemphasised. The TND cases share overlap with other imprinting disorders particularly Beckwith Wiedemann syndrome. Recently the clinical overlap has been proven molecularly and generalised partial hypomethylation of several imprinted loci has been identified. There is accumulating evidence that imprinted genes are part of an imprinted gene network. It is likely that more human imprinting disorders will be recognised as testing for epigenetic mutations becomes more widespread.

Addicted to food? – S12

S12.1

The Selfish brain: competition for energy resources

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The brain takes a primary position in the organism. We present the novel view that the brain gives priority to controlling its own adenosine triphosphate (ATP) concentration. It fulfils this tenet by orchestrating metabolism in the organism. The brain activates an energy-on-request system that directly couples cerebral supply with cerebral need. The request system is hierarchically organized among the cerebral hemispheres, the hypothalamus, and peripheral somatomotor, autonomic-visceromotor, and the neuroendocrine-secretomotor neurons. The system initiates allocative behavior (i.e. allocation of energy from body to brain), ingestive behavior (intake of energy from the immediate environment), or exploratory behavior (foraging in the distant environment). Cerebral projections coordinate all three behavioral strategies in such a way that the brain's energy supply is guaranteed continuously. In an ongoing learning process, the brain's request system adapts to various environmental conditions and stressful challenges. Disruption of a cerebral energy-request pathway is critical to the development of obesity: if the brain fails to receive sufficient energy from the peripheral body, it compensates for the undersupply by increasing energy intake from the immediate environment, leaving the body with a surplus. Obesity develops in the long term.

S12.2

Peptide YY: cure for obesity?

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Exogenous administration of the gut hormone peptide YY3-36 (PYY3-36) reduces food intake in obese humans and rodents. New lines of evidence support a role for endogenous PYY3-36 in regulating energy homeostasis. The NPY-Y2 receptor mediates the anorectic actions of PYY3-36 with rodent studies implicating the hypothalamus, vagus and brainstem as key target sites. Functional imaging in humans has confirmed that PYY3-36 activates brainstem and hypothalamic regions. The greatest effects, however, were observed within the orbitofrontal cortex, a brain region involved in reward processing. Further evidence for a hedonic role for PYY3-36 is supported by rodent studies showing that PYY3-36 decreases the motivation to seek high-fat food. These emerging hedonic effects of PYY3-36 together with the weight-reducing effects observed in obese rodents suggest that targeting the peptide YY system may offer a therapeutic strategy for obesity.

S12.3

Hypothalamic regulation of energy balance

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Obesity and its consequences, such as type-2 diabetes, cardiovascular disease and cancer, are serious health threats. However, despite what the obesity epidemic might suggest, the balance between caloric intake and expenditure is regulated with tremendous precision under most circumstances. Thus, a regulatory system exists, where deviations from the defended body adiposity level trigger signals that can be monitored by specific intracellular metabolic pathways. Such pathways are linked to adaptive responses in energy intake and oxidation, ensuring that body weight remains relatively stable over time. Growing evidence suggests that signals from both currently available fuels (i.e. recently ingested food) and stored fuels derived from the adipose mass converge on brain centers, mostly within the hypothalamus, and act on neuronal targets by modulating intracellular metabolic pathways. Cells use fuel sensing mechanisms to sense ATP levels and to modulate anabolic and catabolic processes. Intriguingly, 'fuel sensors' such as the enzymes AMP-activated protein kinase (AMPK) and target of rapamycin (TOR), have been recently implicated in the regulation of feeding behavior. In particular, our studies focusing on the TOR signaling cascade have demonstrated how such pathway has been co-opted by an important subset of hypothalamic neurons as one sensory input that might affect a variety of metabolic functions. As a consequence, the dysregulation of fuel sensing mechanisms within the hypothalamus may contribute to metabolic dysregulation underlying diseases, such as obesity and type 2 diabetes.

S12.4

Metabolic and reward cues affect the central clock in the brain

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Daily rhythmicity in neuroendocrine functions and sleep-wake cycle is controlled by an endogenous circadian timing system, organized in a network of oscillatory structures. At the top of this circadian multi-oscillatory network, there is a master clock located in the suprachiasmatic nuclei of the hypothalamus. The molecular clockwork involves various clock genes, such as Period (Per).
Light is the most potent synchronizer of the suprachiasmatic clock. By contrast, meal time, as modulated by temporal restricted feeding, has much less synchronizing influence on it, except when animals are no longer exposed to a light–dark cycle. In conditions of constant darkness, a daily restricted feeding can synchronize the circadian rhythms controlled by the suprachiasmatic clock. Moreover, in animals fed with regular food available *ad libitum* and housed in constant darkness, daily reward cues provided by a palatable diet can also entrain the suprachiasmatic clock. This suggests that activation of reward-related network can, to some extent, feedback to the main circadian clock.
Finally, even when animals are exposed to a light–dark cycle, timed calorie restriction (i.e., when only a hypocaloric diet is given each day) is a synchronizer powerful enough to modify the suprachiasmatic clockwork as well as the phase-shifting effects of light. This feedback of metabolic cues to the main clock involves *Per1* and *Per2* genes.
Taken together, these data indicate that both metabolic and reward cues can modulate the synchronization of the central clock.

Pro & Con – HRT or more: Androgen therapy for the aging male – S13

Abstracts Not Required

Basic highlights – S14

S14.1– ESE Young Investigator Award

Direct visualization of cyclic AMP levels in three dimensional cultures of thyroid follicles

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Cyclic AMP (cAMP) is the principal intracellular mediator of TSH effects in the thyroid, inducing both thyroid hormone production and cell proliferation. This notion derives from a large series of evidences, mainly obtained by biochemical approaches on cell lines or primary thyrocytes. However, given the limited resolution in time and space of the techniques employed so far, little is know about the spatial localization and temporal dynamics of cAMP signaling in thyroid cells. Moreover, it is well known that the functional unit of the thyroid is a multicellular structure, i.e. the thyroid follicle. Therefore, a deeper understanding of TSH/cAMP cascade and of thyrocyte biology in general will probably require an *in vivo* approach, either on isolated thyroid follicles or on whole animals. The aim of this study was to develop a method for real-time monitoring of cAMP levels in living thyroid follicles. To this purpose we established a protocol for three dimensional culture of thyroid follicles, which results in good morphology and allows microscopic visualization. To monitor intracellular cAMP levels, we employed a Fluorescence Resonance Energy Transfer based sensor (Epac1-cAMPs), the fluorescence of which is inversely proportional to cAMP levels. Thyroid follicles obtained from a transgenic mouse expressing Epac1-cAMPs were then isolated and cultured as mentioned above. By this approach we were able to evaluate the kinetics of cAMP accumulation and clearance after TSH stimuli of different intensity and duration. The kinetics observed after a short application of TSH were unexpectedly fast, with t1/2 of $\sim$1 and 3 min. for cAMP accumulation and degradation, respectively. These data are consistent with an unpredicted speed of TSH association and dissociation from its receptor. The newly developed method paves the way to a series of *in vivo* studies aimed at further elucidating the spatio-temporal organization of TSH/cAMP signaling cascade.

S14.2

Dopamine receptor 2 activation reduces cells viability in non-functioning pituitary adenomas by inhibiting vascular endothelial growth factor secretion

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Dopamine (DA) therapy of non-functioning pituitary adenomas (NFA) can result in tumor stabilization and shrinkage. However, this effect is not always apparent and the mechanism of action is still unknown. Previous evidence showed that DA inhibits pituitary Vascular Endothelial Growth Factor expression (VEGF), that, in turn, is related to pituitary tumor growth. Our study aimed at clarifying whether VEGF secretion modulation might mediate the effects of DA agonists and chimeric compounds interacting with both DA receptor 2 (DR2) and somatostatin receptor (SSTR) 2 and 5 on cell proliferation in human NFA primary cultures. We assessed DR2, SSTR2 and SSTR5 expression, both at mRNA and protein level, as well as the *in vitro* effects on VEGF secretion and on cell viability of a selective DR2 agonist, cabergoline (Cab), and of the chimeric compound BIM-23A760, which interacts with DR2, SSTR2 and 5. Nine NFA were examined by RT-PCR and by microscopy immunofluorescence for expression of α-subunit, DR2, SSTR2 and 5. Primary cultures were tested with Cab and with BIM-23A760. All NFA samples expressed α-sub, while DR2 and SSTR2 were expressed in 5 and SSTR5 in 2 samples. In DR2 expressing tumors, Cab (but not BIM-23A760) significantly reduced cell viability (-18%; $P<0.05$) and VEGF secretion (-15%; $P<0.05$). These effects were counteracted by treatment with the DA antagonist sulpiride. In addition, the antiproliferative effects of Cab were completely blocked by co-treatment with VEGF. Our data demonstrate that Cab, but not the chimeric SSTR/DR compound, via DR2 can inhibit cell viability by reducing VEGF secretion in a selected group of NFA, providing support for the employment of DA agonists in medical therapy of NFA expressing DR2. Moreover, these data further support the hypothesis that pituitary VEGF expression is under dopaminergic control and underline the importance of an accurate biomolecular analysis of pituitay adenomas.

S14.3 – ESE Young Investigator Award

Variants of the phosphodiesterase 11A (PDE11A4) gene and genetic predisposition to adrenocortical tumors (ACT)

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Introduction
We have previously identified (using a whole-genome large scale SNP- screening approach) germline inactivating stop codon mutations of the PDE11A4 in patients with Cushing syndrome due to micronodular adrenal hyperplasia. PDE11A4 is a cAMP/cGMP phosphodiesterase.
Aim of the study
To investigate the role of PDE11A genetic alterations in a large cohort of ACT.
Materials and methods
Leukocyte DNA from 117 adrenocortical tumors (ACTs) were collected: 45 adrenocortical cancers (ACC), 43 adrenocortical adenomas (ACA) and 29 macronodular adrenal hyperplasia (AIMAH). Leukocyte DNAs from matched volunteers were collected by the same investigators after clinical examination to rule out endocrine tumors (control group). The PDE11A4 20 coding exons (exons 3–23) were amplified by polymerase chain reaction (PCR).
Results
One inactivating mutation was found in one non-secreting ACA (R307X); 21 missense mutations were also found: in 7 out 45 ACC (1 AA showed two differents missense

mutations); in 7 of 29 AIMAHs, in 8 of 43 ACAs; no protein-truncating mutations were found in the controls. Two missense mutations (R804H and R867) were found in 5 ACTs and 5 of 192 controls, respectively; one missense mutation (A349T) was observed in one control. A higher frequency of missense and nonsense mutations in all 3 ACT groups than in controls groups were observed (16% vs 10% in ACC, 19% vs 10% in ACA and 24% vs 9% in AIMAH). This difference was significant in the AIMAH group, with an odds ratio of 3.53 ($P=0.05$). A significant difference between the ACC and the control groups for the synonymous polymorphism in exon 6 (E421E) was also found with an odds ratio of 2.1 ($P=0.05$).
Conclusion
PDE11A missense mutations and the E421E synonymous polymorphism are more frequently found in ACTs than in controls; PDE11A4 plays a role in genetic predisposition to adrenocortical tumorigenesis that extends beyond micronodular hyperplasias with which it was originally associated.

S14.4

Targeted disruption of *Slc2a8* (GLUT8) reduces ATP levels and mitochondrial potential of spermatozoa

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GLUT8 is a class 3 sugar transport facilitator, and transports glucose with high affinity (km ~ 2 mM). GLUT8 mRNA is expressed in brain, heart, skeletal muscle, adipose tissue, adrenal gland, liver and at particulary high levels in testis. In testis, the GLUT8 protein is located in an intracellular compartment of spermatocytes, spermatids and mature spermatozoa. GLUT8 contains an N-terminal dileucine sorting signal retaining the transporter in an intracellular compartment. So far a stimulus inducing translocation of the transporter to the plasma membrane has not been found. Therefore, a function for GLUT8 in intracellular compartments rather than at the plasma membrane is considered. In order to investigate the physiological role of GLUT8, the corresponding *Slc2a8* gene was disrupted in mice. *Slc2a8* knockout mice are viable and appeared healthy. Body weight gain and body composition did not differ between *Slc2a8* knockout and wild-type mice. Analysis of the offspring distribution of heterozygous matings indicated a lower number of *Slc2a8* knockout progeny (30.5: 47.5: 22%, Slc2a8+/+, $Slc2a8^{+/-}$, and $Slc2a8^{-/-}$ mice, respectively) resulting in a significant deviation ($P<0.0024$) from the expected Mendelian distribution. This difference was associated with a significant reduction of ATP levels (0.39 ± 0.11 vs 0.78 ± 0.15 μM/μg protein) and mitochondrial membrane potential (42 ± 4.06 vs 57.3 ± 3.03% orange JC-1 fluorescense). In addition number of motile sperm cells was markedly reduced (31 ± 2.26 vs 60.25 ± 2.09%) in *Slc2a8* knockout as compared with wild-type spermatozoa. In contrast, sperm number, epidydimal maturation, survival rate, intracellular Ca^{2+} levels and glucose uptake were not altered in spermatozoa of $Slc2a8^{-/-}$ mice. These data indicate that GLUT8 plays a role in energy metabolism of sperm cells.

S14.5

KLF11 differentially regulates PDX1-induced activation of the human insulin promoter in rodent beta-cells and human non-beta-cells

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KLF11 is a pancreas enriched member of the Sp1/KLF transcription factor family which plays an important role in mammalian gene regulation. Previously we demonstrated that human (h)KLF11) inhibits human insulin promoter (hInsP) activity in rodent beta-cell lines via an unknown mechanism. Here we present results from ongoing experiments in which we test the hypothesis that KLF11 interferes with the major hInsP transactivator PDX1. We performed transient cotransfections of INS-1E and beta-TC3 beta-cells and HEK293 non-beta-cells with hInsP-controlled SEAP (secreted alcaline phosphatase) reporter plasmids and CMV-driven expression plasmids for hKLF11 and/or hPDX1 at concentrations of 0.5 μg unless otherwise noted. DNA amounts were equilibrated by addition of empty plasmid (mock). As demonstrated before, hKLF11 dose-dependently inhibits -881hInsP activity in both beta-cell lines employed (0.5 μg, 30%; 1 μg, 50%). Ectopic PDX1 only marginally stimulates hInsP (a known effect resulting from the presence of endogenous PDX1 in beta-cells) and, similar to transfections without PDX1, KLF11 inhibits hInsP activation about 30%. In contrast to beta cells, KLF11 stimulates −881hInsP activity in HEK293 about 40%. This stimulation is abolished in shorter hInsP fragments lacking the GC box, a known KLF11 binding site (≤ -355) and converted into 30–40% inhibition in very short hInsP fragments (≤ -101). In hInsP fragments ≤ -101 also PDX1 stimulated activation was nearly abolished, while PDX1 enhances hInsP activation to about 300% in all fragments ≤ -173. Interestingly, the combination of KLF11 and PDX1 superadditively stimulates -881hInsP activity about 10-fold. This superadditive stimulation is continuously reduced to levels of PDX1 alone by deletion of hInsP from −881 to −173. These results strongly indicate that KLF11-mediated inhibition of hInsP activity in beta-cells is closely related to PDX1 function. For further proof of concept we currently investigate physical interactions of KLF11 with PDX1 and its transcription complex partners, for example p300/CBP.

S14.6

Specific neuronal deletion of cannabinoid type 1 receptor (CB1) mice exhibits reduced fat mass and diet-induced obesity resistance

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Aims
Recently, the endocannabinoid system emerged as a pivotal regulator of food intake, ingestive behavior and energy metabolism, acting through CB1 and its endogenous ligands, the endocannabinoids. CB1 antagonists may reduce body weight and improve metabolic profiles in animals and humans by a double mechanism: at first, they target mesolimbic and hypothalamic nuclei and, thereafter, peripheral organs involved in energy storage and expenditure. Nevertheless, it is still unknown how the ECS can exploit its action on these sites and how it can integrate all the outcomes in order to regulate energy balance.
Methods
Firstly, we generated a mouse line ($CB1^{f/f;CaMKIIaCre}$) in which the CB1 gene was conditionally disrupted only at level of principal neurons of the forebrain, including mesolimbic and hypothalamic neuronal populations modulating the incentive value to palatable food and the orexigenic signals, respectively. Then, we measured growth and food intake in conditional and whole knock-out mice (CBN), on standard diet and high fat diet, and assessed body fat distribution by microCT scans. Moreover, we explored several pathways included endocannabinoid biosynthesis/degradation, hormonal secretion, inflammation and fatty acids production by Real-Time PCR in peripheral organs, such as white adipose tissue, liver and skeletal muscle.
Results
Our results showed that both CB1 whole and conditional CB1 knock-out mice were lighter than their control, without affecting food intake. MicroCT scans explained this difference in body weight showing a significant reduction in fat mass of both models and this discrepancy was highlighted in HFD. Moreover, Real-Time PCR analysis showed different gene expression profile between CBN, $CB1^{f/f;CaMKIIaCre}$ mice and their controls, in particular of those genes involved in lipogenic and metabolic pathways, suggesting that other mechanisms may overcome the central CB1 orexigenic effect in maintaining body weight reduced.

GH treatment of syndromic short stature – facts and myths – S15

S15.1

SHOX deficiency: does GH treatment do any good?

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The Short Stature Homeobox-containing gene, SHOX, encodes a transcription factor involved in regulation of growth. *SHOX* haploinsufficient patients including those with Turner syndrome (TS) show growth impairment with or without mesomelic skeletal dysplasia. This study assessed the efficacy of GH in treating short stature associated with SHOX deficiency (SHOX-D). Prepubertal short patients (24 males, 28 females; 3.0–12.3 years) with molecularly-proven SHOX-D were randomized to either a GH-treatment (GH-Tx) group (Humatrope 0.05 mg/kg per d; $n=27$) or an untreated (Un-Tx) control group ($n=25$) for 2

years. For comparison 26 patients with TS (4.5–11.8 years) received GH (GH-Tx TS). Between-group comparisons of height velocity (HV) and height SDS were performed using ANCOVA. GH-Tx SHOX-D patients had significantly greater 1st and 2nd year HV than Un-Tx patients ((mean ± S.E.M.) 8.7 ± 0.3 vs 5.2 ± 0.2 cm/y, 7.3 ± 0.2 vs 5.4 ± 0.2 cm/y, both $P < 0.001$), which was similar to GH-Tx TS patients (8.9 ± 0.4 cm/y, 7.0 ± 0.2 cm/y). GH-Tx patients also had significantly greater 2nd year height SDS (−2.1 ± 0.2 vs −3.0 ± 0.2, $P < 0.001$) than Un-Tx patients. GH treatment did not adversely affect body proportions. In addition, we assessed the relative effect of GH on adult height SDS gain in a separate cohort of patients with SHOX-D and TS, including 14 patients (12 females) with SHOX-D and 158 with TS from various databases. Height SDS gain from baseline was significant for each group (SHOX-D versus TS: 1.1 ± 0.2 vs 1.2 ± 0.1, both $P < 0.001$). Although patients with SHOX-D received a lower GH dose (0.25 vs 0.31 mg/kg per wk, $P = 0.030$) for a 0.9 years shorter duration, there was no significant difference in height SDS gain. We conclude that GH treatment in patients with SHOX-D improves longitudinal growth short-term as well as adult height similarly to patients with TS without adversely affecting body proportions.

S15.2

GH therapy in Noonan syndrome – facts and myths

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Noonan syndrome (NS) is one of the most common non-chromosomal syndromes seen in children with congenital heart disease. Half of them have mutations in the PTPN11 gene, a gene that has a role in modulating cellular proliferation, differentiation, migration and it is required in several developmental processes. Height is not affected at birth, but during childhood short stature is present in about 80% with a less percentage being short at adult age due to delayed bone age and gain in height during pubertal years. The cause of short stature in NS is not clear. Partial skeletal dysplasia may be involved, as in Turners syndrome. Like in Turner, children with NS were thought not to be GH deficient. It is shown that 50% of those studied had mean overnight GH concentrations below the lower limit of the normal range. Moreover, children with NS are found to have high basal levels and wide GH pulse. The majority have low IGF-I levels despite GH status, and it is speculated if these have a failure in the GH postreceptor signaling. High GH levels with low IGF-I are particularly common in children found to have the PTPN11 gene mutation, which also will respond less well to GH treatment.
A considerable number of children have today undergone treatment with GH. The majority of studies have shown similar results. There is a significant increase in growth velocity for the first and second year of GH treatment. The delta height is about 1 SDS after two years of treatment and no dose dependent response is seen. The height growth on GH treatment during pubertal years continues. Studies comparing historical data from Noonan references show an improvement of mean height from start to adult years of 2 SDS in males and 1.4 SDS in females treated with GH. This is of the same magnitude as GH treatment in females with Turner syndrome or short children born small for gestational age.
The potential adverse events of GH treatment are cancer development, insulin resistance and hypertrophic cardiomyopatia. Studies without GH treatment show an increased risk of juvenile myelomonocytic leukemia and it is reported development of lymphoma during GH treatment. Transient increase in insulin but no insulin resistance is reported. Finally, whether GH treatment worsens hypertrophic cardiomyopatia is debated. GH treatment in GH deficient or idiopatic short stature stimulates hypertrophic development of the heart and therefore it is not recommended to start GH treatment if serious heart failure in patients with NS. Moreover, treatment should be monitored regularly concerning IGF-I levels and cardiac function.

S15.3

GH therapy in children with Prader Willi Syndrome (PWS)

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Background
PWS is neurogenetic disorder characterized by muscular hypotonia, hypogonadism, psychomotor delay, obesity, and short stature. Several reports have shown that GH treatment not only results in a remarkable growth, but also in an improvement of body composition and increased muscle strength and agility. Data about the effects of GH-treatment on motor and cognitive abilities and on respiratory parameters in PWS children are limited.
Methods
In a Dutch nation-wide study, 91 children with PWS (42 infants, 49 prepubertal children) were randomized to start with either GH treatment or no treatment for 1 or 2 years, resp. Age range was 0.6–11 years. Next to height, weight, head circumference and body composition by DXA scan, a complete polysomnography (PSG) was performed before and during the study. In addition, tests were performed to assess psychomotor development. Nocturnal sleep and respiration were recorded in a standard fashion. GH dosage was 1 mg/m^2 per day.
Results
Median height SDS increased significantly during GH and did not change in controls. Median fat decreased significantly during GH and increased in controls. Median lean body mass increased significantly during GH and decreased in controls. The baseline Apnea Hypopnea Index (AHI) was 5.5 h (normal range 0–1 h), with very wide inter-individual variations. Of these, 2.9 h were identified as central apneas and 0.8 h were obstructive apneas. No correlation was found between AHI and age or BMI. During GH treatment, the AHI declined with ~50% in the majority of patients, mainly due to a reduction of central apneas. GH significantly improved mental and motor compared to randomized controls.
Conclusion
GH treatment normalizes height SDS and improves body composition in children with PWS. They have a high Apnea Hypopnea Index, mainly due to central apneas. During GH treatment a decline in AHI is found in the majority of patients, due to less central apneas. GH improves psychomotor development. Our results indicate that GH has an important role in PWS, also in young children.

S15.4

Consensus on GH Treatment in SGA

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In 2006, the International Paediatric Endocrine Societies and the GH Research Society convened a meeting to consider the management of the SGA child through to adulthood. This included consideration of strategies for management of the short SGA child who has failed to show catch-up growth. The following statements relate to the outcomes of this meeting.
Short children born SGA form a heterogeneous group with various aetiologies and treatment should be preceded by an effort to identify the diagnosis. Early evaluation of short children born SGA is recommended, and those under 2 years of age with a current length below −2.5 SD should be referred for evaluation. Factors associated with the response to GH over the first 2–3 years include age and height SDS at start of treatment, mid-parental height and dose. Average height gains after 3 years of GH treatment range from 1.2 to 2.0 S.D. for doses of 35 to 70 μg/kg per day. After the initial catch-up, most of this height gain is maintained up to adult height. The discrepancies between the official indications by the FDA in 2001 (age at start 2 years, no height cut-off stipulated, GH dose 70 μg/kg per d) and the EMEA in 2003 (age at start 4 years, height SDS < −2.5, GH dose 35 μg/kg per d) were recognised. It was proposed that SGA children aged between 2 and 4 years who show no evidence of catch-up with a height < −2.5 S.D. should be eligible for GH treatment. With regard to GH dose, it was proposed that the starting dose should cover the range 35 to 70 μg/kg per day with the higher doses used in those with the most marked growth retardation. In the majority of SGA children treated with GH, pubertal timing will be normal, and inhibition of puberty with GnRH analogue treatment is not routinely recommended. There should be a positive response to GH treatment (height velocity SDs > +0.5 in the first year of treatment). If not, re-evaluation is indicated, including consideration of compliance, GH dose, diagnosis and the decision to discontinue treatment. Treatment emergent adverse events are not more common in SGA than in other conditions treated with GH. It is currently unknown whether GH therapy for the SGA subject through childhood and adolescence is associated with benefits or amplification of risks (such as metabolic consequences) in adult life.

New insights in PCOS – S16

S16.1

Definition of PCOS

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Clinical features associated with PCOS include obesity, hirsutism or acne, cycle abnormalities and infertility. Therefore, depending on the primary complaint these patients visit different medical specialists such as general practitioner, pediatrician, dermatologist, medical endocrinologist and gynecologist. The variability in primary complaint asks for different approaches in the work-up of these patients. Hence, doctors usually see just a proportion of the overall spectrum of PCOS and they apply different inclusion criteria for the diagnosis. Still, we all refer to these patients with the same PCOS, causing much confusion.
The 1990 NIH consensus workshop proposed chronic anovulation along with clinical or endocrine signs of hyperandrogenemia as obligatory criteria for PCOS diagnosis. This consensus was recently revised during the 2003 Rotterdam meeting (published simultaneously in both F&S and HR in January 2004), where the occurrence of polycystic ovaries was added to the diagnostic criteria, along with the statement that patients needed to score positive for only 2 out of 3 criteria. This approach (also applied for the diagnosis of the metabolic syndrome) underlines that PCOS concerns a heterogeneous syndrome, with no single feature being mandatory for the diagnosis. It was recognized that that the clinical assessment of hyperandrogenemia is subjective and that good assays to measure free and biologically active testosterone is only available in few laboratories worldwide. Important endocrine features associated with PCOS, including elevated LH and impaired glucose tolerance, are not involved in the diagnosis.

	NIH	NIH extension	Novel phenotypes (Rotterdam criteria)
Oligo/anovulation	+	+	+
Hyperandrogenemia	+	+	+
Polycystic ovaries	+	+	+

For the gynecologist focusing on anovulatory infertility, PCOS is part of the spectrum of WHO type 2 anovulation, characterized by normal serum FSH and E_2 concentrations. It is generally believed that PCOS patients present with poor reproductive outcome following infertility therapies. However, this contention is largely based on retrospective and uncontrolled observations. Recent longitudinal follow up studies from our own group showed that cumulative singleton birth rates up to 75% can be achieved with conventional approaches for ovulation induction (involving clomiphene citrate as first line, and exogenous gonadotropinsd as second line) followed by IVF. Recent novel approaches for ovulation induction such as insulin sensitizing agents, aromatase inhibitors, laparoscopic ovarian surgery procedures and IVF involving single ET all need further evaluation in PCOS.
PCOS women frequently present with metabolic abnormalities such as insulin resistance, dislipidemia and hypertension, even at young age. Therefore, currently much attention is shifted towards the assessment of increased health risks of PCOS women later in life, such as type 2 diabetes and cardiovascular disease. Unfortunately, sufficiently powered studies with a sufficient duration of follow-up are extremely scare so far.

S16.2

New insights in polycystic ovary syndrome

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Polycystic ovary syndrome (PCOS) is a common disorder often emerging post-menarche, and hallmarked by androgen excess and a low ovulation rate. Less consistently, PCOS is characterized by hyperinsulinemic insulin resistance, dyslipidemia, increased abdominal adiposity, low-grade inflammation, and the presence of polycystic ovaries (PCO) on ultrasound.
At least two developmental pathways seem to lead to PCOS. One begins with a normal prenatal growth and continues, via simple obesity, to an absolute fat excess; the other begins with fetal growth restraint, continues in infancy with rapid catch-up of weight into the upper-normal range, and leads to relative adiposity at age 4 year, and to visceral fat excess at age 6 year. In late childhood and puberty, both pathways may converge to conform 'pre-PCOS', a state characterized by hyperinsulinemia, adiposity, low-grade inflammation (as reflected by an increased neutrophile-to-lymphocyte ratio and decreased concentrations of total and high molecular weight adiponectin), amplified adrenarche (with or without precocious pubarche), and early-normal puberty and menarche, which may result in a decreased final stature. Finally, the two pathways diverge again, the 'postnatal-overweight' pathway rather leading to PCOS-with-PCO, while the 'prenatal-underweight' pathway is more often linked to an adult PCOS phenotype with glucose intolerance and without PCO.
In conclusion, markers have been identified along two pathways to PCOS. These markers allow for timely recognition of 'pre-PCOS' and for intervention, with overweight-control and/or insulin sensitization. The early origins of PCOS may thus be pivotal in the development of the adult phenotype, and partly determine the heterogenity in the clinical presentation.

S16.3

Vascular markers in PCOS

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PCOS, the commonest endocrinopathy among reproductive-aged women, lies to the core of intensive research due to the multiplicity of its pathophysiologic and clinical aspects. Among the most intensively investigated fields,the clustering of metabolic abnormalities and cardiovascular risk factors in PCOS has been widely acknowledged. Functional, biochemical and morphological markers of subclinical cardiovascular disease have been explored in PCOS women. On balance the weight of evidence does not unveil clinically pronounced abnormalities, but points to the accelerated onset of vascular structural and functional lesions of in PCOS women. There is a growing body of literature identifying traditional vascular risk factors, mainly central adiposity, dyslipidemia, glucose intolerance, and diabetes in women with PCOS. Taken into account that these abnormalities are encountered as early as the second decade of life in patients with PCOS, these subjects represent significantly higher risk to develop cardiovascular incidents, in comparison to their age and BMI matched peers. Added to the detrimental metabolic profile, PCOS is characterized by low-grade systemic inflammation, as indicated by elevated serum levels of C-reactive protein and inflammatory cytokines (i.e. IL-6 and IL-18), increased leucocyte count, increased levels of Advanced Glycation End Products and amplified oxidative stress. Increased serum levels of the adhesion molecules sVCAM-1 and sE-selectin in women with PCOS reflect low-grade chronic inflammation of the endothelium. Increased levels of endothelin-1 and decreased flow-mediated dilatation,as determined by ultrasonography in conduit arteries, both indicate abnormal vascular tone, involved in the course of atherosclerosis.Morphological studies, in women with PCOS compared with matched controls, have been indicated by increased carotid wall thickness on B-mode ultrasonography and increased scores of coronary/aortic calcification on electron beam computed tomography.However, reflective of the pathophysiologic and clinical heterogeneity of PCOS,metabolic disturbances and vascular dysfunction are not universal findings in women with the syndrome.
Most importantly,risk factors and surrogate markers of subclinical cardiovascular disease have not been proved by epidemiological,mainly retrospective, studies to be translated into overt clinical disease or events. Prospective studies are required to address the end point of the cardiovascular events in this high-risk population. The course of cardiovascular abnormalities which starts from vascular risk factors and proceeds to subclinical disease, awaits further exploration in PCOS.

S16.4

Treatment of PCOS

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Polycystic ovary syndrome (PCOS) is a extremely common endocrinopathy, occurring in 5–7 percent of women in reproductive age. PCOS, a hyperandrogenic disorder, is the most common cause of infertility in women. The diagnosis of PCOS has life-long implications with increased risk for infertility, metabolic syndrome, diabetes mellitus type 2, and possibly cardiovascular disease. The recent criteria outlined in the EHSHRE/ARM consensus statement ('Rotterdam criteria') have been proposed to make the diagnosis of PCOS (two out of three of the following criteria): oligo- and/or anovulation, clinical and/or biochemical signs of hyperandrogenism, polycystic ovaries (by ultrasound). Other causes of hyperandrogenism, such as an androgen secreting ovarian tumor, hyperprolactinemia, or NC CAH, should be excluded. Polycystic ovary syndrome (PCOS) must

be suspected in every adolescent girl with menstrual irregularity, hirsutism, obesity, persistent acne vulgaris, scalp hair loss and hyperhidrosis. Treatment for PCOS is still as controversial as difficult: oral contraceptive pill OCP (increasing concentrations of SHBG while decreasing androgen secretion, it reduces FAI), spironolactone and drospirenone, an analogue of spironolactone, progestin, cyproterone acetate, glucocorticoid therapy (beneficial in the treatment of menstrual irregularity in the minority of nonobese PCOS with a strong component of FAH), GnRH agonist therapy, flutamide/nilutamide (specific but hepatotoxic antiandrogen with efficacy similar to that of cyproterone), finasteride (a type 1 5-alpha-reductase inhibitor), metformin (improving insulin sensitivity, suppressing appetite and enhancing weight loss, promoting ovulation) and thiazolidinediones (?). Weight loss and every treatment reducing insulin resistance in both obese and lean women with PCOS may have beneficial results causing a fall in ovarian androgen secretion and an improvement in pituitary-ovarian axis, which may have impact on hair growth, menstrual regularity and fertility. Physical hair removal to mask the presence of excess hair are basic to treat hirsutism: chemical depilitating agents, bleaching, waxing techniques, laser therapy and electrolysis.

Thyroid cell biology – S17

S17.1

Basic mechanisms of thyroid development

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Tissue-specific transcriptional regulation is achieved by the combined action of transcription factors, co-regulators and components of the basal transcriptional machinery. The goal of our work is to unravel the mechanisms involved in the regulation of the activity of thyroid-specific transcription factors. We have demonstrated that the transcription factor Pax8 is required for the expression of all the thyroid-specific genes that are considered markers of the differentiated phenotype of this cell type. Moreover, we have recently shown that Pax8 biochemically interacts with TTF-1, another transcription factor involved in thyroid gene expression. Interestingly, the physical interaction between the two factors leads to a strong synergistic effect on the transcriptional activation of a thyroid-specific gene promoter. Although Pax8 and TTF-1 influence the expression of various genes during mouse embryonic development, their mode of action as transcription factors remain poorly understood.
TAZ is a transcriptional coactivator that regulates the activity of several transcription factors therefore playing a central role in tissue-specific transcription. The recently demonstrated physical and functional interaction between TAZ and TTF-1 in the lung raised the question of whether TAZ could be an important regulatory molecule also in the thyroid. In our study, we demonstrated the presence of TAZ in thyroid cells and the existence of an important cooperation between TAZ and the transcription factors Pax8 and TTF-1 in the modulation of thyroid gene expression. In addition, we revealed that the three proteins are co-expressed in the nucleus of differentiated thyroid cells and that TAZ interacts with both Pax8 and TTF-1, *in vitro* and *in vivo*. More importantly, we show that this interaction leads to a significant enhancement of the transcriptional activity of Pax8 and TTF-1 on the thyroglobulin promoter thus suggesting a fundamental role of TAZ in the control of genes involved in thyroid development and differentiation.

S17.2

Growth factors involved in thyroid cells differentiation

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Cell differentiation occurs as a result of the concerted activation of multiple signalling pathways in response to environmental stimuli. We have stu died the role elicited by several growth factors and oncogenes in the control of Sodium/Iodide Symporter (NIS) gene expression, a thyroid differentiation marker. Our results have shown that IGF1/PI3K and TGFbeta/Smads inhibit NIS gene transcription induced by TSH/cAMP, a mechanism mediated by the inhibition of Pax8 binding to NIS enhancer. Concerning oncogenes, we have shown that BRAFV600E, the most frequent genetic event in thyroid cancer, sharply impaired both NIS expression and targeting to membrane and, surprisingly, this impairment was not totally dependent on the MEK-ERK pathway. Interestingly, we have show that BRAF-induced NIS repression is dependent on the operation of an autocrine loop involving TGFbeta, whose secretion was induced by BRAFV600E. Subsequently, inhibition of TGFβ-receptor or its kinase activity clearly reinduced NIS functional activity in thyroid cells cancer. We conclude that the crosstalk and cooperation between multiple pathways may have important implications in thyroid differentiation, providing new insights of the mechanisms underlying tumorigenesis.

S17.3

Molecular mechanisms of thyroid carcinogenesis: role of the CBX7 gene: Loss of the CBX7 gene.ll

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Using gene expression profiling we found that the CBX7 gene was drastically down-regulated in six thyroid carcinoma cell lines versus control cells. This suggested that CBX7 is an oncosuppressor. The aim of this study was to determine whether CBX7 is related to thyroid cancer phenotype, and to try to identify new tools for the diagnosis and prognosis of thyroid cancer. We thus evaluated CBX7 expression in various snap-frozen and paraffin-embedded thyroid carcinoma tissues of different degrees of malignancy by quantitative RT-PCR and immunohistochemistry, respectively. CBX7 expression progressively decreased with malignancy grade and neoplasia stage. Indeed, it decreased in an increasing percentage of cases going from benign adenomas to papillary (PTC), follicular and anaplastic (ATC) thyroid carcinomas. This finding coincides with results obtained in rat and mouse models of thyroid carcinogenesis. CBX7 loss of heterozygosity occurred in 36.8% of PTC and in 68.7% of ATC. Restoration of CBX7 expression in thyroid cancer cells reduced growth rate, which indicates that CBX7 plays a critical role in the regulation of transformed thyroid cell proliferation. In conclusion, loss of CBX7 expression correlates with a highly malignant phenotype in thyroid cancer patients. Consequently, CBX7 monitoring could contribute to thyroid cancer diagnosis and prognosis.

S17.4

Novel insights on the Na^+/I^- symporter (NIS): it mediates electroneutral active transport of the environmental pollutant perchlorate

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The Na^+/I^- symporter (NIS) is a key plasma membrane protein that mediates active I^- uptake in the thyroid, lactating breast, and other tissues with an electrogenic stoichiometry of 2 Na^+ per I^-. In the thyroid, NIS-mediated I^- uptake is the first step in the biosynthesis of the iodine-containing thyroid hormones, which are essential early in life for proper development of the central nervous system. In the lactating breast, NIS mediates the translocation of I^- to the milk, thus supplying this essential anion to the nursing newborn. Perchlorate (ClO_4^-) is a well-known competitive inhibitor of NIS. Exposure to food and water contaminated with ClO_4^- is common in the US population and the public health impact of such exposure is currently being intensely debated. Settling the controversy on whether ClO_4^- is a NIS blocker or a transported substrate of NIS, we show *in vitro* and *in vivo* that: NIS actively transports ClO_4^-, including ClO_4^- translocation to the milk; a simple mathematical fluxes model accurately predicts the effect of ClO_4^- transport on the rate and extent of I^- accumulation; and, strikingly, the Na^+/ClO_4^- transport stoichiometry is electroneutral, uncovering that NIS translocates different substrates with different stoichiometries, an unprecedented finding for any transporter. That NIS actively concentrates ClO_4^- in the maternal milk suggests that exposure of newborns to high levels of ClO_4^- may pose a greater health risk than previously acknowledged, as ClO_4^- would thus directly inhibit the newborns' thyroidal I^- uptake. In addition, we have generated mutant NIS proteins that transport ClO_4^- electrogenically.

Doping – an issue for clinical endocrinologists – S18

S18.1

Indirect evidence suggesting hormone abuse: can it be proof of doping in sports?

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Men and women who compete in sporting events at the international level perform a tremendous volume of exercise training. This training is necessary to promote the high level of adaptations in the musculoskeletal, cardiovascular and neuroendocrine systems which are required in order to improve human performance. The incentives to perform such rigorous and demanding exercise training, and compete well in sporting activities, are substantial. An Olympic medal can result in large financial rewards to an athlete and allow for opportunities to gain even further remuneration. Regrettably, such 'high-stakes' incentives can entice some athletes to seek unethical and illegal means of improving their performance. The continuing high-profile occurrences of doping (i.e., hormonal abuse) in sports throughout the last few decades only underscore how persuasive such 'enhancement' can be to athletes. The current prevalence of doping in sports is an issue of debate, but it may be on the rise in athletes throughout the world (i.e., both professionals and amateurs who compete at international levels as well as the lower ranks of club sports). However, the ability to chemically screen for doping of all athletes is logistically unfeasible. Thus the question of whether 'indirect evidence' of doping can accurately and reliably suggest the existence of abuses has been raised by sports governing bodies. This presentation will address this question and also whether indirect evidence may be an effective means to screen/detect for doping. This presentation will be particularly relevant to clinicians who have opportunities to encounter athletes in their medical practices.

S18.2

Doping with androgens

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Anabolic androgenic steroids (AAS) are known to be misused both in competitive and in non-competive sports. As AAS stimulate protein synthesis in muscle cells, athletes expect performance-enhancing effects beyond that brought about by training alone. In 1974 the IOC and the International Amateur Athletic Federation (IAAF) first banned the use of AAS. This prohibition encompassed only synthetic steroids, such as metandienone, stanozolol etc. and the misuse of endogenous steroids, e.g. testosterone, was not restricted. As AAS are not used directly during competition but rather during training to increase muscle strength, athletes stopped administration of AAS before competition, switching to AAS such as testosterone, which were not banned at that time. Based on results from the Olympic Games 1980 in Moscow testosterone was banned in 1984.

To control the misuse of synthetic AAS nowadays urine samples of athletes collected after competition events or out of competition were tested by gas chromatography in combination with mass spectrometry (GC/MS).

The control of testosterone misuse is rather difficult as the identification of this androgen by MS is not sufficient to report a doping offence. An analytical method to prove testosterone abuse has unambiguously to distinguish between endogenous production and exogenous application of testosterone. This challenge was successfully solved by quantitative analysis of urinary excreted testosterone in comparison to its isomer epitestosterone (testosterone/epitestosterone quotient). Elevated T/E-ratios are considered as suspicious for testosterone misuse. Such biological samples are further analysed by isotope ratio mass spectrometry (IRMS) of carbon 13C/12C which allows to verify the origin of testosterone and its main metabolites.

S18.3

Distinction between endogenous and exogenous GH

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Recombinant human growth hormone (rhGH) is abused as a performance enhancing drug, but our knowledge about this is mainly based on rumours, confessions of former athletes and on actions of the police. For many years, the detection of rhGH application by biochemical methods was thought to be impossible. Physiological and biochemical properties of hGH made the development of a test method difficult. rhGH is identical in amino acid sequence and chemical properties to the main, 22 kD isoform of endogenous GH. Half life in circulation is extremely short, and concentrations are highly variable between and within individuals due to pulsatile secretion and influence of factors like stress, sleep and nutrition. Thus, quantification of GH alone is not a reliable measure to decide about the origin of the GH molecules in a sample. To develop a test, two independent approaches are investigated: One is based on the analysis of changes in GH dependent parameters in blood. Application of rhGH induces an increase in IGF-I, IGFBPs and markers of bone and cartilage turnover which exceeds normal fluctuation and is more pronounced than that seen after physiological elevation of circulation GH. A combination of markers allows identification of athletes treated with rhGH, provided the appropriate reference ranges for each hormone are available. The second approach is based on analysis of the GH isoform composition of a sample. A wide spectrum of homo- and heterodimers of different GH isoforms is secreted by the pituitary, whereas rhGH is monomeric 22 kDa GH only. After application of rhGH the 22 kDa isoform becomes predominant, and this can be demonstrated by specific immunoassays. Such assays have been validated in large studies and tested during the Athens and Torino Olympic games. Their routine use in anti doping laboratories now is established to allow regular out of competition testing.

S18.4

Distinction between endogenous and exogenous erythropoietin (EPO)

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Sports authorities prohibited the use of EPO in 1988 and at present any analogue or mimetic is also included in the list of prohibited substances from the World Anti-Doping Agency (WADA). The presentation will summarize the main analytical strategies developed to identify the presence of recombinant erythropoietin (EPO) administered as a doping agent.

Indirect evidence is based on the analysis of blood parameters (haemoglobin, haematocrit, reticulocytes, macrocytes, etc.) and serum markers (concentration of EPO and serum transferrin receptors, etc). The problem of inter-technique comparison for reliable results evaluation will be emphasized, especially for serum markers.

Charge differences between isoforms of recombinant EPO and native urinary EPO are the grounds for the isoelectric focusing–double blotting–chemi-luminescence detection method presently approved for doping control. Its advantages and limitations will be presented and commented on. The chemical bases of the differential detection will be highlighted and some future approaches for detection also presented.

The appearance and detectability of EPO analogues and mimetics susceptible for abuse will also be addressed.

Hormone receptors and adipose tissue – S19

S19.1

The family of corticotropin-releasing hormone (CRH) peptides: important regulators of adipocyte function

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Through activation of the HPA axis, corticotropin-releasing hormone (CRH), the 41-amino acid hypothalamic peptide, plays a fundamental role in mammalian survival and response to stressful stimuli. CRH belongs to a family of stress-related peptides that includes urocortins (UCNs) that have been linked with many pathophysiological effects. Their actions are mediated via activation of two types of CRH receptors (CRH-R1 and R2) that exhibit distinct pharmacology and functional properties. Emerging evidence points towards important actions for CRH and UCNs in the regulation of energy balance and homeostasis. Central actions of these peptides control food uptake and exert potent anorectic and thermogenic effects. Moreover, new potential

peripheral sites of CRH and UCNs actions have also been described; CRH and UCNs directly regulate skeletal muscle thermogenesis as well as insulin signalling whereas UCN-III modulates pancreatic insulin secretion. *In vivo* animal models and cellular studies have identified CRH and UCNs as important regulators of adipose tissue biology. CRH-Rs are expressed in both white and brown adipocytes. In white adipocytes, CRH peptides modulate 11β-HSD1 activity and cellular lipolysis, suggesting direct regulation of cortisol/cortisone levels. CRH also exerts *in vivo* effects on femoral adipose tissue metabolism, since i.v. administration leads to increased levels of interstitial and plasma glycerol suggesting stimulated lipolysis. Both CRH-R1 and R2 receptors are expressed in mouse interscapular brown adipocytes and in cellular models of brown adipocytes (T37i cell line). In the latter example, CRH and UCNs stimulate glycerol release and lipolysis through activation of the cAMP/PKA signalling pathway. This pathway also involves phosphorylation of hormone sensitive lipase (HSL) and redistribution of lipid–droplet-specific proteins such as perillipin. In brown adipocytes, UCN-II is also a potent activator of other signalling pathways such as ERK1/2 and p38MAPK, raising the possibility for diverse biological effects. Although the molecular and physiological characteristics of the adipocyte CRH-R system are still poorly characterised, these studies provide strong evidence for an important role for CRH /UCN-driven metabolic effects, important for maintenance of central stress pathways regulating energy homeostasis and adaptive responses to environmental perturbations. Important for maintenance of central stress pathways regulating energy homeostasis and adaptive responses to environmental perturbations.

S19.2

Corticosteroid receptors and adipocytes

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Adipose tissue is central to the control of energy and glucose homeostasis. Modulation of corticosteroid action in adipose tissue represents a potent mechanism to alter this homeostasis. Corticosteroid hormone action in adipocytes has long been considered to be mediated by glucocorticoid receptors (GR). We and others have recently identified the mineralocorticoid receptor (MR) as a new target to modulate adipocyte function. The generation of homo- and heterodimers of the GR and the MR is essential for mediating corticosteroid actions in tissues expressing both receptor types. Using newly generated murine GR- and MR-knock-out and knock-down adipose lines from white and brown fat depots we have recently aimed at dissecting specific roles of corticosteroid receptors for controlling key adipose functions including adipogenesis, adipokine expression profiles, insulin sensitivity, and transdifferentiation from lipid-storing white towards thermogenic brown adipocytes. Recent data from our and other groups support the hypothesis that the MR is essential for adipogenesis. An imbalance between MR and GR action, e.g. in the presence of chronic selective GR stimulation or via a GR knock-down, appears to promote the development of a large white cell phenotype or predominantly pro-inflammatory responses, respectively. A better understanding of the specific MR and GR roles may, in analogy to selective estrogen receptor modulators (SERMs), contribute to the development of new therapeutic options to prevent and treat the metabolic syndrome and its cardiovascular complications.

S19.3

Multi-layered receptor interactions in ghrelin-induced fat deposition

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Binding of the gastric hormone ghrelin to its cognate receptor, GHS-R_{1a}, is dependent upon the acylation of its third (serine) residue. Although acute exposure to ghrelin stimulates the secretion of growth hormone (a potent lipolytic signal), chronic exposure to ghrelin promotes fat deposition[1].

We have shown that in intra-abdominal depots, ghrelin-induced fat accumulation is depot-specific, time-dependent, due to lipid accumulation resulting from suppressed lipolysis and is probably mediated by GHS-R_{1a} in adipocytes[2,3]. Like other G protein-coupled receptors (CPCRs), it is becoming evident that activation of GHS-R_{1a} may be modulated by heterodimerization with other GPCR species and by regulation of intracellular signalling components[4]. These processes may underlie the depot-specific sensitivity of adipose tissue to ghrelin. Although inguinal fat (a deep subcutaneous depot) exhibits many of these ghrelin-induced responses, superficial subcutaneous adipose tissue is unaffected by exposure to ghrelin[2,3]. In bone marrow, however, ghrelin increases adiposity by a direct induction of adipogenesis. This increase in adipocyte number is unlikely to be mediated by GHS-R_{1a}, since it is also induced by unacylated ghrelin but not by the GHS-R_{1a}-specific ligand L163 255[2].

In physiological terms, continuous exposure to elevated circulating ghrelin, which signifies prolonged nutritional insufficiency, appears to exert depot-specific gating of lipid utilization, whilst ghrelin and UAG promote the accumulation of adipocytes in bone marrow by an unknown receptor.

References
1. Tschöp M *et al*. 2000 *Nature* **407** 908–913.
2. Thompson NM *et al*. 2004 *Endocrinology* **145** 234–242.
3. Davies JS *et al*. 2007 *Proceedings of the 89th Annual Meeting of the Endocrine Society* Ab P1–31.
4. Davies JS *et al*. 2006 *Proceedings of the 88th Annual Meeting of the Endocrine Society* Ab P3–118.

S19.4

Adiponectin and its receptors in insulin resistance, diabetes, and metabolic syndrome, and obesity

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Adiponectin is a major insulin sensitizing hormone, which activates AMP kinase and PPARα pathways to facilitate glucose and lipid metabolism to increase insulin sensitivity. Decreased plasma adiponectin linked to obesity is causally involved in insulin resistance and metabolic syndrome in obesity. Moreover, decreased plasma adiponectin is causally involved in atherosclerosis. In fact, decreased plasma adiponectin levels have been shown to be associated with future development of diabetes and cardiovascular diseases in humans. These biological effects of adiponectin are mediated via adiponectin receptors, AdipoR1 and AdipoR2. Activation of AdipoR1 by adiponectin stimulates AMP kinase pathway and that of AdipoR2 by adiponectin stimulates PPARα pathway, thereby ameliorating insulin resistance. AdipoR1 and AdipoR2 are down-regulated in obesity, which is also casually involved in insulin resistance linked to obesity. PPARγ agonists upregulate plasma adiponectin levels, which contributes to amelioration of insulin resistance and atherosclerosis. On the other hand, PPARα agonists upregulate adiponectin receptors. Recently, adiponectin has been shown to stimulate AMPK activity in the arcuate hypothalamus and increase food intake via AdipoR1. Moreover, adiponectin decreases energy expenditure. Adiponectin appears to serve as a starvation gene. Thus adiponectin is upregulated according to decreased white adipose tissue mass, which then centrally facillitates energy storage and peopherally facillitates fatty acid combustion to generate energy for survival. In times of plenty, adiponectin is downregulated according to increased white adipose tissue mass, namely obesity, thereby causing insulin resistance, diabetes, and the metabolic syndrome. Thus, adiponectin and adiponectin receptors are crucially involved in the development of insulin resistance, diabetes, metabolic syndrome, and obesity and thus may serve as molecular targets of treatment and prevention strategy of these diseases.

References
1) *Nat Med* 2001 **7** 941, 2) *Nat Med* 2002 **8** 856, 3) *J Biol Chem* 2002 **277** 25863–25866, 4) *J Biol Chem* 2003 **278** 2461–2468, 5) *Nature* 2003 **423** 762, 6) *J Biol Chem* 2004 **279** 30817–30822, 7) *Mol Cell* 2005 **17** 171, 8) *J Biol Chem* 2006 **281** 8748–8755, 9) *J Clin Invest* 2006 **116** 1784–1792, 10) *Nat Med* 2007 **13** 332–339, 11) *Cell Metab* 2007 **6** 55–68.

Translational highlights – S20

S20.1 ESE Young Investigator Award

sst2 gene transfer restores the somatostatin agonist sensitivity of human resistant pituitary adenomas, *in vitro*

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Somatotroph adenoma represent 20% of pituitary adenoma. Although often considered as benign, they can induce serious neurological and metabolic complications. GH hypersecretion can be inhibited by somatostatin agonists such as octreotide or lanreotide. However, some GH levels reach normal values in only 50% of treated patiens. The decreased sensitivity to the somatostatin agonists was shown to be related to a lower expression of the sst2 gene.
To reverse the octreotide resistance, our aim was to reintroduce sst2 gene in human pituitary adenoma cells *in vitro*. Sst2 gene transfer was done by adenoviral vector (Ad-sst2). Adenoviral vector expressing eGFP (Ad-eGFP) was used as control vector. The transgene sst2 expression was followed by real-time PCR and by immunocytochemistry. The sst2 mRNA expression increased to 100 fold after infection. In resistant tumors, the sst2 immunostaining was observed at the membrane in the transduced cells whereas the sst2 staining was intracellular in the control cells. In somatotroph adenoma cells, sst2 gene transfer induced not only a decrease in hormonal secretion but also a decrease in cell number by an apoptotic effect, using a caspase-dependant pathway. The decrease in GH secretion was due, at least in part to the decrease in hormonal expression. Five days after 5MOI adenoviral infection, sst2 gene transfer improved the octreotide sensitivity. The dose-related inhibition of GH release was moved toward the lower concentrations. A decrease in EC50 was observed (about 1 log) in all tumours. Moreover, the maximal GH suppression under octreotide increased in resistant ones. In conclusion, sst2 gene may constitute a new therapeutic gene for somatotroph adenoma resistant to the somatostatin agonists.

S20.2

SF-1 knockout mice as a model for hormone independent brain sexual differentiation

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Animals that are not exposed to endogenous sex steroids during development provide an important model for studying hormone independent development of brain sex differences. Due to gonadal agenesis, male and female steroidogenic factor 1 knockout (SF-1 KO) mice are born phenotypically female. Normally, they die shortly after birth due to adrenal insufficiency. Early corticosteroid injections followed by adrenal transplantation can maintain SF-1 KO mice into adulthood. As several aspects of hypothalamic structure and function do not become apparent until after puberty, examination of brains from adult SF-1 KO mice can provide unique information.
Vasopressin (AVP) neurons in the bed nucleus of the stria terminalis (BNST) show sex differences, with males having more cells and a denser projection to the lateral septum than females. This sex difference was reported as partially hormone independent with a contribution from the presence of sex chromosomes. In the present study, we examined AVP immunoreactive fibers in the lateral septum in SF-1 KO mice to further clarify the extent to which the sex difference is hormone independent or influenced by developmental exposure to sex steroid hormones. After two weeks of treatment with testosterone prior to sacrifice, AVP immunoreactive fibers in the lateral septum of SF-1 KO and prepubertally gonadectomized WT control mice were less than expected, suggesting that exposure to sex steroids over a long period is necessary to maintain normal adult levels of AVP expression in the BNST to lateral septum projection. Statistical analyses did not reveal sex differences in AVP immunoreactive fiber density; however, there were significantly fewer AVP immunoreactive fibers in male SF-1 KO mice, suggesting that exposure to testosterone during the perinatal period is at least partially responsible for the greater AVP expression in WT male mice.

S20.3

Monoallelic mutations in DUOXA2 are associated with mild permanent hypothyroidism and goiter

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Generation of H_2O_2 at the apical membrane of thyroid cells is essential for iodination of thyroglobulin. Dual oxidase 2 (DUOX2) is the catalytic core of the thyroidal H_2O_2 generator, and its deficiency leads to congenital hypothyroidism (CH) in humans and mice. The Dual oxidase maturation factor 2 (DUOXA2) is a recently identified endoplasmic reticulum (ER)-resident protein required for expression of DUOX2 activity.
Aims
We aimed the identification of human DUOXA2 defects as a novel cause of dyshormonogenetic thyroid disease.
Patients and methods
Eighty-three CH patients with partial and total iodide organification defects were screened for mutations in the coding regions of DUOX2 and DUOXA2 genes.
Results
Two missense mutations, W132L and G294E, were identified in DUOXA2. Mutations are heterozygous and are located in exons 4 and 6 of the gene, encoding a long intra-ER loop, predicted to interact with DUOX2, and the cytoplasmatic carboxy-terminal tail of the protein, respectively. Cotransfection of DUOX2 and DUOXA2 cDNAs in mammalian COS cells shows that W132L and G294E reduce *in vitro* H_2O_2 production capacity to 32% and 16% of wild type DUOXA2, respectively.
W132L was identified in an European Caucasian girl diagnosed with non-goitrous CH, showing TSH of 25 mUI/l at screening and 152 mUI/l at serum confirmation with FreeT4 of 10.5 pmol/l. Reevaluation at 3 years of age showed progressive increase of TSH and T4 treatment was re-introduced. G294E is present in an American-Hispanic girl not subjected to CH screening, consulting at 12 years of age for short stature. She was diagnosed with a large euthyroid goiter, with positive perchlorate test discharge of 24%, and T4 substitution was implemented. DUOX2 gene showed no pathogenic mutations in these patients.
Conclusions
DUOXA2 monoallelic defects are associated with mild but permanent hypothyroidism and /or goiter. DUOXA2 is a novel candidate gene for inheritable dyshormonogenetic thyroid defects.

S20.4

Recent advances in stereological methods for studying microvessels in endocrine organs

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Blood and lymphatic microvessels (MVs) ensure a supply of substances to the organ cells and removal of catabolic wastes into circulation. In endocrine glands, MVs are also involved in transport of hormonal molecules to systemic blood. These functions of MVs are vitally important, and many investigators study MVs in endocrine organs, including thyroid gland.
Stereological methods are frequently used to determine 3D features of MVs (number, sizes, density, etc.) on their images of lower dimension, usually 2D sectioning profiles. Without these methods, much valuable information can be lost about correlations between structure and function in endocrine glands.
We review stereological methods designed recently by our company for studying MVs. These methods include: (i) the method for defining 3D MV sizes, with ellipticity of the MVs being taken into consideration, (ii) the method for estimating the MV angular distributions in 3D space, and (iii) the method for determining MV spatial arrangement pattern based on the second-order stereology parameter (pair correlation function). All these methods meet

to the requirements of novelty and inventive step used in patenting procedure (patents RU2211487, RU2218601, RU2219583; application RU2003131039, procedure passed). The methods were applied in the study of thyroid blood MVs. The thyroid perifollicular capillaries were revealed to be elliptical (typical 3D axial ratio was equal to 1.6), with their angular distributions being isotropic (Krasnoperov & Gerasimov *Exp Biol Med* 2003 **228** (1) 84–92). Spatial arrangement of the capillaries was inhibitory, so that the capillaries tend to avoid each other in the gland volume (Krasnoperov & Stoyan *Ann Biomed Eng* 2006 **34** (5) 810–822). The designed set of the stereological methods is instrumental in microanatomy-based studying of microcirculatory networks physiology in endocrine organs.

S20.5

V206M polymorphism of the SLC26A6 gene encoding a Cl^--oxalate transporter in patients with primary hyperparathyroidism and kidney stones

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Primary hyperparathyroidism (PHPT) is associated with increased risk of kidney stones. Hypercalciuria and urine oxalate excretion are considered risk factors for urolithiasis in PHPT stone-formers. Recently, the anion-exchanger SLC26A6 has been involved in the oxalate metabolism. *Slc26a6*-null mice showed hyperoxalemia, hyperoxaluria resulting in dramatic calcium oxalate urolithiasis. We tested the hypothesis that urine oxalate excretion in PHPT patients might be modulated by polymorphic variants of the human SLC26A6 gene. A well-characterized PHPT cohort ($n=145$) and 129 age- and sex-matched healthy subjects were genotyped by TaqMan allelic discrimination system. The homozygous 206V genotype was the most frequent both in PHPT patients and healthy controls (79.3% and 74.4%, respectively), while heterozygosity for the 206M allele was detected in 20.0% and 23.3%, respectively. Due to the rarity of the 206M allele, homozygous and heterozygous patients were grouped in V/M+M/M set. The prevalence of kidney stone disease did not differ between the V/V and V/M+M/M group as well as the severity of hyperparathyroidism. Nonetheless, the 206M alleles were more frequently associated with higher urine oxalate excretion than the median level (77.7% vs 41.5%, $P=0.008$). Considering the subset of PHPT stone formers ($n=68$), though the V/V group did not differ from the V/M+M/M group for age, sex and severity of hyperparathyroidism, urine calcium excretion levels were lower in V/M+M/M PHPT stone formers with respect to V/V stone formers (4.40 ± 1.88 vs 5.92 ± 2.62 mg/kg per 24 h; $P=0.034$). Finally, the 206M alleles were related to the presence of hypertension (73.3 vs 47.8%, $P=0.022$), showing an OR for hypertension of 4.2 (95% IC 1.5–12.1, $P=0.008$). In conclusion, these findings suggested a potential role of SLC26A6 206M polymorphism in kidney stones development in PHPT patients, since it was associated with urolithiasis in the presence of relatively low urinary calcium excretion. Moreover, it was associated with high prevalence of hypertension.

S20.6

A functional AMH polymorphism is associated with follicle number and androgen levels in polycystic ovary syndrome patients

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Polycystic ovary syndrome (PCOS) is characterized by anovulation, elevated levels of circulating androgens and polycystic ovaries. Although the etiology of PCOS is poorly understood, the common denominator is a disturbance in the selection of the dominant follicle. In PCOS women serum Anti-Müllerian Hormone (AMH) levels are elevated. Since AMH reduces FSH sensitivity of growing follicles, the elevated AMH levels in PCOS patients may contribute to the disturbed follicle selection and elevated androgen levels. We investigated the role of AMH signaling in the pathophysiology of PCOS using a genetic approach. The study was approved by the local Medical Ethics Review Committee.

A frequent polymorphism in the AMH gene (AMH $Ile^{49}Ser$) was studied in a large cohort of PCOS women ($n=331$). A cohort of 32 normo-ovulatory women and a population-based cohort of 3635 postmenopausal women (the Rotterdam study) served as controls. Genotype and allele frequencies for the AMH $Ile^{49}Ser$ polymorphism were similar in PCOS women and controls (MAF=0.20). However, within the group of PCOS women, carriers of the AMH ^{49}Ser allele had less often polycystic ovaries (OR=0.05, 95% CI=0.01–0.40, $P=0.006$) compared to non-carriers. Furthermore, AMH ^{49}Ser allele carriers had lower testosterone and androstenedione levels (nmol/l) compared to non-carriers (1.84 ± 0.08 vs 2.03 ± 0.06, $P=0.05$, and 11.9 ± 0.4 vs 13.0 ± 0.3, $P=0.04$, respectively). Serum AMH, FSH, LH and estradiol levels were not different between the genotype groups. Furthermore, *in vitro* studies demonstrated that the bioactivity of the AMH ^{49}Ser protein is diminished compared to the AMH ^{49}Ile protein ($P<0.0001$). In conclusion, genetic variants in the AMH gene do not influence PCOS susceptibility. However, the association of the AMH $Ile^{49}Ser$ polymorphism with follicle growth and androgen levels suggests that AMH contributes to the phenotype of PCOS.

Pro & Con – Is there a benefit for treatment of subclinical hypo? – S21

Abstracts Not Required

The good side of exercise – S22

S22.1

Mechanisms for the delayed effects of exercise on lipid and lipoprotein metabolism

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Increased plasma triglyceride (TG) concentration is an important risk factor for cardiovascular disease. Regular aerobic exercise is associated with lower plasma TG concentrations and lower cardiovascular risk. The cardioprotective effect of endurance-type physical activity is therefore likely, to some extent at least, linked to the hypotriglyceridemic effect of aerobic exercise. It has long been recognized that the TG-lowering effect of exercise manifests in response to a single bout of aerobic activity with little, if any, evidence for chronic physiologic adaptations after repeated exercise sessions, i.e., training.

Results from several recent studies investigating the metabolism of very low density lipoproteins (VLDL) by using stable isotope labeled tracers have shed light on the mechanisms whereby exercise lowers plasma TG concentrations. Prolonged exercise of moderate intensity (2 h at 60% of maximal oxygen consumption), performed in the evening, lowers plasma and VLDL-TG concentrations the next morning ($\sim$15 h after exercise cessation). This is due to increased VLDL-TG plasma clearance rate, i.e., accelerated removal of VLDL-TG from the circulation, without any changes in VLDL-TG secretion rate by the liver. On the contrary, the hepatic secretion of VLDL-apolipoprotein B-100 (apoB-100) is suppressed by exercise. This suggests secretion of fewer but TG-richer VLDL particles after exercise, which may facilitate their intravascular hydrolysis by lipoprotein lipase. Interestingly, exercise fails to affect VLDL-TG secretion by the liver even though it greatly enhances free fatty acid (FFA) availability, i.e., the major precursor for hepatic TG synthesis, as indicated by the much higher FFA concentration and FFA rate of appearance in plasma after exercise, which could not be matched by the smaller increase in fatty acid oxidation rate.

The effects of exercise leading to hypotriglyceridemia are dose-dependent, since moderate-duration exercise (1 h at 60% of maximal oxygen consumption) does not affect VLDL-TG and VLDL-apoB-100 concentrations and kinetics. Nevertheless, such exercise is still sufficient to promote a great

increase in FFA concentration and FFA rate of appearance in plasma, with no concomitant increase in fatty acid oxidation, clearly indicating that under these circumstances, FFA availability in plasma is not a major determinant of VLDL-TG secretion by the liver.
Evidence also suggests that systemic factors considered important for the regulation of VLDL-TG, VLDL-apoB-100, and plasma FFA metabolism, such as insulin, growth hormone, cytokines and adipokines, do not appear to mediate the effects of exercise on hepatic VLDL metabolism. Likewise, sex (men or women), the mode of aerobic exercise (running or cycling), and the type of exercise (endurance or resistance) do not emerge as important regulators of VLDL-TG and VLDL-apoB-100 metabolism response to exercise.
Collectively, recent data indicates that exercise-induced hypotriglyceridemia results from increased removal rate of VLDL-TG from the circulation, which may be facilitated by the secretion of fewer but TG-richer (and therefore likely larger) VLDL particles after exercise. The major determinant of this response appears to be the energy expenditure of exercise, whereas the role of traditional regulators of hepatic VLDL metabolism, such as insulin and the availability of plasma FFA, remains obscure.

S22.2

The good side of exercise: inflammation and physical activity
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Chronic low-grade systemic inflammation is a feature of chronic diseases such as cardiovascular disease and type 2 diabetes. Regular exercise offers protection against all-cause mortality, primarily by protection against atherosclerosis and insulin resistance and there is evidence that physical training is effective as a treatment in patients with chronic heart diseases and type 2 diabetes. Regular exercise induces anti-inflammatory actions. During exercise, IL-6 (interleukin-6) is produced by muscle fibres. IL-6 stimulates the appearance in the circulation of other anti-inflammatory cytokines such as IL-1ra (interleukin-1 receptor antagonist) and IL-10 (interleukin-10) and inhibits the production of the pro-inflammatory cytokine TNF-alpha (tumour necrosis factor-alpha). In addition, IL-6 enhances lipid turnover, stimulating lipolysis as well as fat oxidation. It is suggested that regular exercise induces suppression of TNF-alpha and thereby offers protection against TNF-alpha-induced insulin resistance. Recently, IL-6 was introduced as the first myokine, defined as a cytokine, that is produced and released by contracting skeletal muscle fibres, exerting its effects in other organs of the body. Myokines may be involved in mediating the beneficial health effects against chronic diseases associated with low-grade inflammation such as diabetes.

S22.3

Why does physical exercise improve insulin sensitivity, the role of adipose tissue?
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Modern lifestyle diminished significantly the need of everyday exercise, which is probably one of the major factors leading to the development of several metabolic diseases including type 2 diabetes, hypertension and atherosclerosis with its deadly consequences. Epidemiological studies proved that higher levels of cardio-respiratory fitness are associated with lower mortality irrespective of fat stores. Physical activity reduces all-cause, cardiovascular as well as cancer associated mortality. Beneficial effects of exercise on insulin sensitivity have been demonstrated in a short term as well as following a longer periods of physical activity. Short-term effects are detectable even after a single bout of exercise and are mediated mostly by the metabolic changes in insulin signalling and substrate fluxes inside the muscle tissue. Especially modifications in fatty acid metabolism related to decreased intracellular accumulation of intermediary metabolites interfering with insulin signalling have strong impact on improved muscle insulin sensitivity. In a longer perspective, physical activity modifies gene expression of key proteins involved in a regulation of insulin signalling, glucose transport and substrate metabolism in muscle (GLUT4, glycogen synthesis) leading to an improved glucose tolerance. Moreover, regular physical activity has been shown to change the metabolism of adipose tissue. Adipose tissue is potent endocrine organ producing several proteins collectively called 'adipokines', several of which regulate insulin sensitivity in a negative while others in a positive way. Modification of gene expression and production of adipokines represents another possibility, how exercise improves insulin sensitivity, decrease pro-inflammatory state and mediate its wide spread beneficial effects. Other factors, like changes in sympathetic nervous activity or endothelial dysfunction might also be involved.

S22.4

Exercise and bone
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Osteocytes are the most abundant cell type in bone; however, they remain the least characterised of these. Several theories have been proposed regarding their function, including osteolysis, sensing the strains produced in response to mechanical loading of bones and producing signals that affect the function of osteoblasts and osteoclasts as well as the expression of molecules that directly affect the turnover process. This review also discusses the role of osteocyte apoptosis in targeted bone remodeling and proposes that the incidence of osteocyte apoptosis is in line with the description of apoptosis as an essential homeostatic mechanism for the healthy maintenance of tissues.
Inappropriate targeting of bone remodeling underlies a number of musculoskeletal disease states including Paget's disease and Osteoporosis. The mechanism driving the targeting of bone resorption is unknown. Our previous studies have demonstrated a close association between regions of bone containing apoptotic osteocytes and resorption surfaces in both healthy and pathological conditions. Such findings raise the possibility of a currently unknown causal signaling mechanism between the dying osteocyte and effector cells at the bone surface.
Identification of the specific signals implicated in this phenomenon and the method by which they are delivered will profoundly increase our understanding of the effector cell targeting system in bone providing a platform to production of novel intervention strategies.

Towards a better understanding of hypothalamic–pituitary disorders – S23

S23.1

Pituitary development and congenital hypopituitarism (CH)
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Normal hypothalamo-pituitary development is critically dependent upon a complex genetic cascade that dictates organ commitment, cell differentiation, and cell proliferation within the anterior pituitary. Mutations in a number of transcription factors have been implicated in the aetiology of congenital hypopituitarism (CH) in mice and humans; resulting phenotypes and their inheritance may be highly variable. Mutations in genes implicated in early pituitary development may be associated with variable extra-pituitary phenotypes e.g., dominant and recessive mutations in *HESX1* may be associated with septo-optic dysplasia (SOD), combined pituitary hormone deficiency (CPHD) and isolated growth hormone deficiency (IGHD). Duplications and mutations within SOX3 have recently been described in association with infundibular hypoplasia, hypopituitarism and variable mental retardation, whilst *SOX2* mutations are associated with variable CH in association with learning difficulties, oesophageal atresia and anophthalmia. Recessive *LHX3* mutations are associated with CH and a short stiff neck. Dominant *LHX4* mutations are also associated with CH and pointed cerebellar tonsils on MRI. Recessive mutations within the pituitary-specific transcription factor *Prophet of Pit1* or *PROP1* are associated with CH, often with an enlarged pituitary. ACTH deficiency can evolve in a number of patients, reflecting the need for constant review of the phenotype. Dominant or recessive mutations within *POU1F1* are associated with GH, variable TSH and prolactin deficiency. To conclude, genetic analysis together with functional analysis of the mutations at the protein level will in the future have a greater role to play in understanding the mechanisms leading to particular CH phenotypes and their evolution. However, there is no substitute for careful delineation of the clinical, biochemical and neuroradiological phenotype prior to undertaking genetic studies.

S23.2

Paracrinicity and adaptation to hormonal needs

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Although the notion of paracrine and autocrine signalling was already suggested more than 100 years ago, its full recognition dates from the last 30 years. Paracrine communication and autocrine loops have been shown to operate in all hormonal cell types and in folliculo-stellate cells and other non-hormonal cells during fetal and postnatal development and adulthood. More than 100 compounds have been identified that have, or may have, paracrine or autocrine actions, including the neurotransmitters acetylcholine and γ-aminobutyric acid, neuropeptides, growth factors, cytokines, annexin-1, follistatin, hormones, nitric oxide, purines, retinoids and fatty acid derivatives. Connective tissue cells, endothelial cells and vascular pericytes may contribute to paracrinicity by delivering growth factors, heparan sulphate proteoglycans and proteases and basement membranes may influence paracrine signalling through the binding of signalling molecules to heparan sulphate proteoglycans. The present symposium will highlight some of the more recent achievements and growing concepts concerning the integrated action of these factors in maintaining adaptation and homeostasis of pituitary function when hormonal outputs need to be adapted to changing demands of the organism, such as during reproduction, stress, inflammation, starvation and circadian rhythms. A second focus will be the growing notion that paracrine/autocrine actions are highly context-dependent and that specificity and selectivity in these interactions may rely on microanatomical specialisations, functional compartmentalisation in receptor–ligand distribution and the non-equilibrium dynamics of the receptor–ligand interactions in the loops.

S23.3

Growth factors in pituitary tumour development

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Despite considerable progress, the pathogenesis of pituitary tumours is largely unknown. Aberrant expression of oncogenes or tumour suppressors is thought to play a role in pituitary tumour initiation. The originating tumour cells may express abnormal patterns of growth factor receptors and may release excessive amounts of growth factors or angiogenic factors, which contribute in auto-/paracrine manner to the different speed and degree of progression of pituitary tumours. Herein the expression, pathophysiological function and mechanism of action of some selected cytokines and growth or angiogenic factors such as IL-6, BMP-4, EGF, and VEGF is presented and the tumourigenic potential of these factors is discussed. The factors act through different classes of receptors (receptors without enzymatic activity, Ser/Thr kinase receptors, Tyrosin kinase receptors) and induce different signalling cascades. Therefore, it is speculated whether blocking the interaction of the growth factors with their receptors or inhibiting components of the intracellular signalling cascades may provide tools for novel pharmacological treatment concepts of pituitary tumours.

S23.4

Cell cycle control and proliferative diseases of the pituitary

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The importance of cell cycle regulation in pituitary biology has been suggested from the fact that many gene-targeted mouse models of cell cycle mutations develop diverse pituitary pathologies. The retinoblastoma protein (pRb)/cyclin-dependent kinase 4 (Cdk4) pathway, one of the major mitogen-sensor routes in the cell, seems to be critical in the control of pituitary cell proliferation. Both inactivation of pRb or hyperactivation of Cdk4 result in pituitary hyperplasias or tumors *in vivo*. In addition, the cell cycle inhibitor p27Kip1 also plays crucial roles in maintaining pituitary homeostasis and p27Kip1 mutations strongly cooperate with the alteration of the pRb/Cdk4 pathway. In fact, these molecules or their regulators are frequently altered by both genetic and epigenetic mechanisms in pituitary tumors as well as in other endocrine pathologies. The use of gene-targeted mouse models has allowed us to dissect *in vivo* the molecular mechanisms behind these mutations. These data is also suggesting potential therapeutic strategies as some of these molecules, such as Cdks, are 'druggable' targets. The analysis of these mutations in specific cells *in vivo* also provides a valuable tool to understand the role of cell cycle regulation in specific pituitary progenitors or putative pituitary stem cells. Thus, whereas most hyperplasias or adenomas are formed of differentiated, hormone-expressing cells, some aggressive pituitary tumors observed in Cdk4/p27Kip1 double mutant mice present small undifferentiated cells positive for stem cell markers. These *in vivo* studies using mouse models are therefore suggesting that a proper balance between proliferation and differentiation in specific progenitor cells may be necessary to maintain pituitary homeostasis and the deregulation of key cell cycle proteins may be responsible for specific pituitary pathologies.

State of the art in the therapy of pituitary disease – S24

S24.1

Nonfunctioning pituitary macroadenomas: treatment and follow-up

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Nonfunctioning pituitary macroadenomas are benign tumors, characterized by the clinical and biochemical absence of hormonal overproduction. Clinical symptoms are mainly caused by mass effects of the tumor. The main symptoms are pituitary insufficiencies, visual field defects, decreased visual acuity and headache. For nonfunctioning adenomas with a diameter of less than one cm no treatment is necessary. In patients with a nonfunctioning macroadenoma without visual field defects, the approach can be expectative in individual cases. Transsphenoidal surgery is indicated in patients with visual field defects. Improvement of visual field defects is achieved the majority of patients after surgery. This improvement continues until 1 year after surgery. In contrast to visual function, pituitary function is not likely to be restored after transsphenoidal surgery. Because the adenomas have the propensity for regrowth, after primary treatment life long follow-up is necessary. Until now, there is no reliable tumor-marker for nonfunctioning pituitary tumors. For that reason MRI is the method of choice during long-term follow-up. The assessment of visual field defects is a sensitive method for the detection of tumor growth only when the tumor is close to the chiasm. Because of the propensity of growth, sometimes postoperative prophylactic radiotherapy is advocated. However, even radiotherapy does not prevent tumor regrowth in all cases and adequate tumor control can be achieved by transsphenoidal surgery alone. A strategy without postoperative radiotherapy will prevent that a large number of patients will be exposed to the long-term sequelae of radiotherapy without having any benefit. In treated patients with a nonfunctioning pituitary adenoma, a decreased quality of life has been reported, probably due to the intrinsic imperfection of hormonal replacement therapy.

S24.2

Medical therapy of Cushing's disease

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The first-line therapy of Cushing's disease (CD) is pituitary surgery, whereas pituitary radiotherapy and bilateral adrenalectomy represent alternative treatments for patients not cured by surgery. Medical therapy has a minor role in the management of CD, and it is mainly based on the use of two different categories of drugs, the adrenal-blocking drugs, which act at the adrenal level, and the neuromodulatory drugs, which act at the pituitary level. These drugs are not usually effective as sole long-term treatment of the disorder, and are used mainly either in preparation for surgery or as adjunctive treatment after surgery and/or radiotherapy, waiting for their definitive effectiveness. Among the adrenal-blocking drugs, the most commonly used agent is ketoconazole, which has a rapid onset of action, but it is frequently associated with loss of control of hypercortisolism, a phenomenon known as escape, and is affected by gastrointestinal side-effects, including a liver dysfunction, which rarely induce a severe hepatitis with acute liver failure. The neuromodulatory drugs include a long series of agents which, however, have been never demonstrated a great effectiveness to be routinely used in the management of CD. Recently, the peroxisome proliferators activated receptor γ agonists were demonstrated to induce short-term control of cortisol, with later escape. The possible role of dopamine agonists has been reconsidered in the treatment of CD as short-term treatment with cabergoline was demonstrated to normalize cortisol secretion in 40% of patients with CD. Preliminary data on long-term treatment suggested that more than one third of patient is controlled by cabergoline. The possible role of

somatostatin analogues has been also re-evaluated in the treatment of CD as a newer somatostatin analogue, pasireotide has been demonstrated to inhibit cortisol secretion in a subgroup of patients with CD. Combination treatments with dopamine agonists and specific somatostatin analogues or low-dose ketoconazole might represent effective treatment for CD.

S24.3

The sequealae of Cushing's disease

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Cushing's disease (CD) is often severe and debilitating and is caused by a POMC-expressing corticotrope tumour autonomously secreting ACTH to cause chronic hypercortisolism. Untreated CD is associated with excess mortality. The mainstay of management remains transsphenoidal surgery, but the overall long-term remission rate is a disappointing 55–60%. Compared to surgery for other states of pituitary hormone hypersecretion, there is an excess of new hypopituitarism, itself associated with excess mortality. Bilateral adrenalectomy may be needed to control disease but is associated with the risk of corticotrope tumour progression and Nelson's syndrome. Pituitary radiotherapy delivered by any means is associated with hypopituitarism. Thus, overall management of hypercortisolism is not optimal, and improvements are needed to specifically control ACTH secretion.
What is surprising is the fact that some of the adverse effects of hypercortisolism persist even if remission is achieved. Even after a microadenomectomy the HPA axis is often suppressed for months to years (and occasionally permanently) with risk of adrenal insufficiency and need for glucocorticoid replacement. Recent molecular data suggest that part of the on-going HPA axis suppression is mediated by continued inhibition of *POMC* expression and its transcription factors by histone deacetylation. Excess mortality of CD appears to return to normal after remission, but several data show that cardiovascular risk remains, with a persisting metabolic syndrome characterized by insulin resistance. Osteoporosis appears to improve, but the effects of vertebral fracture remain. The improvement in hypercortisolaemia can also herald an increase in autoimmune disease. Whilst psychological impairment tends to improve in many, for some the most debilitating features are the persistence of cognitive and psychiatric disturbance and significantly impaired Quality of Life.

S24.4

Development of a disease-related QoL-questionnaire for Cushing's disease

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Chronic exposure to hypercortisolism significantly impacts on patient's health and health-related quality of life (HRQoL). We developed a disease-generated questio-nnaire to evaluate HRQoL in Cushing's syndrome (CS) (CushingQoL); in 125 (104 females) patients recruited in Spain, France, Germany, The Netherlands and Italy, clinical and hormonal data were collected and correlated with results of the generic SF-36 questionnaire, a question on self-perceived general health status and the CushingQoL score. 39 (31%) were currently hypercortisolemic and 47 (38%) adrenal insufficient. Psychometric evaluation was adequate: good feasibility (94%), reliability (Crohnbach's alpha = 0.87) and validity (unidimensionality and after Rasch analysis lead to a final version with 12 items). A significant ($P < 0.001$) correlation was observed between CushingQoL score and patients self-perceived general health and SF-36 (Pearson's correlation coefficient > 0.597). Patients with hypercortisolism (defined as increased 24-h urinary free cortisol) scored worse (lower) than those without ($44 + 22$ vs $56 + 21$, $P = 0.004$). Linear regression analysis identified female gender and hypercortisolism as predictors for worse QoL. Conclusion: CushingQoL is useful to evaluate HRQoL in patients with CS and correlates with clinical parameters. Conducted but not funded by ERCUSYN.

Stem cell and regeneration – S25

S25.1

Generation and patterning of patient-specific pluripotent stem cells

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Cellular transplantation has enormous potential for treating a variety of degenerative or malignant conditions. While adult stem cells display limitations in expansion capacity and developmental potential, embryonic stem (ES) cells are an inexhaustable source of tissues for research and therapy. During the last decade, human ES cells have been isolated, and differentiated to several tissue-specific progenitors. *In vitro* differentiating ES cells recapitulate early steps of embryogenesis and often activate pathways that are conserved between mammals and developmental model systems such as fish and frog. Thus, exposure to morphogen gradients mimicking the embryonic environemnt can be exploited to pattern specific fate choices (e.g. blood), from *in vitro* differentiating ES cells.
However, immune barriers hinder the transplantation of ES-derived cellular therapies. Different strategies have been proposed for providing HLA-compatible ('customized') pluripotent stem cells: 1) the establishment of HLA-matched ES cell banks, the generation of 2) genetically identical nuclear transfer ES cells or 3) histocompatible parthenogenetic ES cells, as well as, most recently, the generation of 4) induced pluripotent stem cells (iPS cells) via somatic cell reprogramming with defined genetic factors.
The generation of patient-specific autologous pluripotent stem cells may provide the opportunity to combine gene therapy with autologous cell therapy in the treatment of human genetic disease. Precise *in situ* gene repair can be performed via homologous recombination in cultured cells, followed by autologous tissue transplantation. This approach would circumvent the risks of insertional mutagenesis with viral vectors, as well as of Graft-Versus-Host-Disease following allogeneic transplantation. Although proof of principle experiments have been succesfully performed in murine model systems, a number of technical hurdles needs to be solved before human therapies based on pluripotent stem cells will enter clinical studies.

S25.2

Stem cells in the gonads: novel options for fertility preservation?

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Male and female germline development during sexual differentiation differs significantly. While in the testicular microenvironment primordial germ cells differentiate into a stem cell, the spermatogonium, the female pathway leads to the establishment of a limited pool of oocytes arrested in prophase of meiosis. Recently, exciting research has improved the understanding of pathways involved in germ cell differentiation and maintenance of pluripotency. *In vitro* and *in situ* manipulation will offer novel approaches to create germ cells from pluripotent precursors. The existence of spermatogonial stem cells in the testis offers clinically relevant options for preservation and restoration of male fertility. We have developed approaches to infuse germ cells into rodent and primate testes and showed that germ cell transplantation and testicular grafting are procedures to generate sperm. The rapid progress in the development of novel experimental strategies encourages cryopreservation of gonadal cells and tissues.

S25.3

The cell biology of neural stem and progenitor cells

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Our group studies the cell biological mechanisms of neurogenesis in the context of mammalian brain evolution, specifically the proliferation versus differentiation

of neuroepithelial (NE) cells and their progeny. In the course of neurogenesis, mouse NE cells down-regulate a number of epithelial features. Expression of the anti-proliferative gene Tis21 can be used as a tool to distinguish between proliferating and neuron-generating NE cells. Time-lapse microscopy of neuron-generating divisions of NE cells using transgenic mouse embryos expressing GFP under the control of the Tis21 promoter reveals the existence of a novel neuronal progenitor dividing at the basal side of the neuroepithelium. To study the distribution, during mitosis, of cellular components in the context of the apico-basal axis of NE cells, we focus on prominin-1/CD133, a pentaspan membrane protein sorted to the apical surface of NE cells and specifically retained in plasma membrane protrusions. Prominin-1 is associated with a novel, cholesterol-based membrane microdomain which is involved in prominin's retention in plasma membrane protrusions. Using prominin-1 to define the apical surface of NE cells, we investigate the symmetric versus asymmetric distribution of the apical plasma membrane during proliferative versus neuron-generating divisions of NE cells. Knock-down of Aspm, implicated in microcephaly, by RNA interference in the developing mouse embryo demonstrates that this mitotic spindle pole-associated protein is crucial for maintaining a cleavage plane orientation that allows symmetric, proliferative divisions of NE cells during brain development. Remarkably, preceding the switch to neurogenesis, the prominin-1–containing apical plasma membrane microdomain of NE cells is released into the neural tube lumen as novel extracellular vesicles that originate from the midbody and primary cilium.

Götz & Huttner *Nat Rev Mol Cell Biol* 2005 **6** 777–788.
Huttner & Kosodo *Curr Opin Cell Biol* 2005 **17** 648–657.
Fish *et al*. *PNAS* 2006 **103** 10438–10443.
Dubreuil *et al*. *J Cell Biol* 2007 **176** 483–495.

S25.4

Beta cell progenitor niche(s) and derivation of such cells from human ES cells

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The pancreas originates from the definitive endoderm-derived gut epithelium. Much has been learnt about the specification of the gut endoderm into pancreatic progenitors and their progenies, the endocrine (islets of Langerhans) and exocrine cells, by various transcription factors. However, less is known about the extracellular cues that regulate the expression of such transcription factors. Human ES cells have emerged as a potential tool for studying human pancreas development and as a source for islet cells in cell replacement therapy of diabetes. Progress in identification and characterization of niche(s) important for beta cell development and the use of different strategies for differentiating human ES cells into endoderm and pancreatic cell lineages will be discussed.

New therapeutic options in diabetes – S26

S26.1

Islet transplantation

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The morbidity and mortality associated with long-term diabetic complications continue to rise and will remain a major worldwide health problem in the coming years. Islet cell transplantation has recently emerged as one the most promising therapeutic approaches to improving glycometabolic control in diabetic patients and, in many cases, to obtaining insulin independence. Unfortunately, many flaws still persist that make it impossible for islet transplantation to become the gold standard treatment for type 1 diabetic patients. Investigators are still debating whether islet transplantation should be considered an option limited to specific single cases. We review the state of the art of islet transplantation, the outcomes, the immunosuppression, and – most important – the impact on patients' survival and long-term diabetic complications and eventual alternative options. Finally, we review the many problems surrounding the field and the obstacles that islets face after transplantation. Islet cell transplantation requires a relatively short hospital stay and has the advantage of being a relatively noninvasive procedure. The rate of insulin independence 1 year after islet cell transplantation has significantly improved in recent years (60% at 1 year post-transplantation compared with 15% in past years). Recent data indicate that restoration of insulin secretion after islet cell transplantation is associated with an improvement in quality of life, with a reduction in hypoglycemic episodes and potentially with a reduction in long-term diabetic complications. Once clinical islet transplantation has been successfully established, this treatment could even be offered to diabetic patients long before the onset of diabetic complications.

S26.2

Plasticity of human pancreatic beta cells

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Understanding how beta-cells maintain themselves in the adult pancreas is important for the development of strategies aimed at ameliorating or ideally curing different forms of diabetes. There has been much debate over whether beta-cell proliferation, as a means of self-renewal, predominates compared to the existence and differentiation of a pancreatic stem cell or progenitor cell population. Based on studies in the mouse, both principles can be demonstrated, although in normal physiological conditions beta-cell proliferation is the dominant mechanism. However, it is not clear how well these rodent studies can be extrapolated to human physiology. Beta-cell proliferation is extremely limited in human and there are several lines of evidence suggesting that islet neogenesis may be more prominent mechanism in the human than in the rodent pancreas. The plasticity of human beta cells may be greater than expected. However, induction of beta-cell differentiation *in vitro* remains a major challenge. Our recent observations on the physiological microenvironment of human islets may provide keys to solve this problem.

S26.3

Emerging therapeutic approaches to preserve beta cell function in type 1 DM

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Antibodies to CD3 are potent immunosuppressants now applied as non Fc-receptor (FcR) binding monoclonals.
Data from our laboratory demonstrated that in NOD mice CD3 antibodies could reverse recent onset disease by restoring tolerance to beta cell antigens in a durable fashion. Thus in mice presenting full-blown diabetes, a five consecutive day treatment with low doses of the hamster anti-CD3 monoclonal antibody 145 2C11 or its F(ab)′2 fragments induced complete and durable disease remission, within 2–4 weeks in the absence of insulin treatment. Concerning their mode of action, data from the NOD mouse model indicated that CD3 antibodies promote 1) immediate clearance of insulitis followed by 2) 'resetting' of specialized subsets of immunoregulatory CD4+T cells mediating active tolerance similar to those that control the onset of spontaneous diabetes and 3) recovery of sensitivity of pathogenic T cells to immunoregulation. More recent results showed that the CD3-induced immunoregulatory T cells concentrate within both the CD4+CD25+ and CD4+CD62L+ compartments. We also obtained evidence in support of a key role for the cytokine TGF-beta in the anti-CD3-induced T cell-mediated immunoregulation.
These results have led to clinical trials in recent onset type 1 diabetic patients using non Fc-receptor (FcR) binding monoclonals antibodies to CD3 that are well tolerated since they are devoid of the mitogenic activity that was a hallmark of first generation CD3 antibodies such as OKT3. The results of these studies will be presented and discussed in the context of the current strategies aimed at inducing/restoring immune tolerance in the clinic to preserve over long term beta cell function in type 1 diabetes.

S26.4

The one and only? Gene driving Type2 DM
P Froguel

Abstract unavailable

Nodules and more: new aspects of thyroid disorders – S27

S27.1

Management of thyroid nodules: the European and the USA way
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In 2006, three major society-endorsed guidelines and consensus reports on the diagnosis and management of thyroid nodules were published by the American Association of Clinical Endocrinologists/Associazone Medici Endocrinologi (AACE/AME), the American Thyroid Association (ATA) and the European Thyroid Association (ETA). Despite many similarities and areas with lack of evidence, significant differences between these guidelines are also present, reflecting differences in disease epidemiologies, practice patterns, interpretation of existing data, and the availability of resources in different regions. Some of these differences have been highlighted by several recent surveys among members of these organisations.
The major areas of disagreement between the guidelines concern the indication for scintigraphy, TSH cut off values, the use of fine needle aspiration cytology and of calcitonin determination in the differential diagnosis of thyroid nodules and the role of ultrasound in the follow up and treatment of benign thyroid nodules.
These guideline differences currently do contribute to confusion among practicing endocrinologists. However, they should also be used to further focus and advance our discussion of diagnostic and therapeutic strategies, the outline of future studies and also the elaboration of international guidelines for the diagnosis and treatment of thyroid nodules.

S27.2

Management of differentiated thyroid cancer
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The initial management of differentiated thyroid cancer is based on total thyroidectomy and radioiodine ablative therapy. Less radical treatment is advocate only in unifocal microcarcinomas. Following initial treatment, long term follow-up is initiated, aimed to the early discovery of persistent or recurrent disease. Follow-up strategies has changed in recent years, after the introduction in the clinical practice of neck ultrasound and recombinant human TSH (rhTSH). The drug has been developed as an alternative to thyroid hormone withdrawal in those circumstances when TSH stimulation is needed. In initial clinical trials the drug has been very effective in patients on l-thyroxine suppressive therapy. Two daily 0.9-mg injections stimulate thyroidal ^{131}I uptake and thyroglobulin (Tg) secretion to a degree equal to 2–3 weeks of hormone withdrawal. Several independent works have confirmed the efficacy of rhTSH-based follow-up in clinical practice. Based on these findings it is proposed that the follow-up of DTC patients may consist of periodical serum Tg measurement (in AbTg negative patients) and 131-I uptake after stimulation with rhTSH, with the aim of selecting patients with persistent disease to be submitted to the more appropriate treatment. In addition, using rhTSH, serum Tg measurement is more sensitive than diagnostic WBS in detecting residual disease and the routine use of diagnostic WBS has been questioned. In particular, the results of rhTSH-stimulated Tg combined with the results of neck ultrasonography has the highest diagnostic accuracy, near to 100%, in detecting patients with residual disease. Altogether the available evidence is sufficient to propose a diagnostic follow-up of DTC patients based mainly on the use of rhTSH-stimulated serum Tg and neck ultrasound. Such an attitude will preserve the patients' quality of life by avoiding hypothyroidism and will save many unnecessary diagnostic WBS, reducing the need for imaging and 131-I WBS to the minority of patients with strong suspicion of residual disease.
A second important application of rhTSH is the preparation of patients undergoing post-surgical 131-iodine thyroid ablation. Recent prospective trials, have shown that thyroid ablation with 100 mCi or 50 mCi of radioiodine have similar rates of successful ablation in patients prepared with rhTSH and in those in whom thyroid hormone was withdrawn. Dosimetric studies showed that a further advantage of using rhTSH was a one-third reduction in the radiation dose delivered to the blood. Based on these studies, rhTSH has been approved in Europe and USA for post-operative thyroid ablation.
In conclusion, the introduction of rhTSH and neck ultrasonography has greatly facilitated the current protocol for the management of differentiated thyroid cancer patients both in the diagnostic and therapeutic setting.

S27.3

Therapeutic options and dilemmas in medullary thyroid carcinoma
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Analysis of RET germline mutations, now a routine element of diagnostic algorithm in medullary thyroid carcinoma (MTC), influences follow up of MTC patients but at present has less impact on treatment modalities, which are the same in hereditary and sporadic disease. Microarray analyses confirm that molecular profile is rather similar in both MTC forms.
In hereditary MTC, RET mutation screening in families at risk allows to find asymptomatic mutation carriers, for whom prophylactic thyroidectomy is to be offered. Still, some questions need consideration, among them:
- Should routine RET diagnostics in patients diagnosed with apparently sporadic MTC, behind the known hotspots in exons 10,11 and 13–16, involve all other mutations detected until now?
- Do genotype-phenotype differences justify a delay in prophylactic thyroidectomies in some families?
- What are the legal consequences of RET germline mutation detection?

If diagnosis of MTC is done at later stage, surgery is often unsuccessful and treatment of disseminated disease becomes the main problem both in hereditary and in sporadic MTC. Neither radiotherapy nor classical chemotherapy constitute successful therapy options. Thus, the recent introduction of new targeted therapy modalities has raised much interest. Before all, the use of tyrosine kinase inhibitors (TKI) is being considered. Each TKI has its own spectrum of target kinases and their potency to inhibit angiogenesis is especially important. It is well known that VEGFR expression is increased in MTC and contributes to its aggressiveness, thus, inhibition of VEGFR kinases offers a strong anti-cancer effect. RET protein itself is also a receptor tyrosine kinase, targeted by some TKI. Numerous phase 2 or phase 3 trials have been initiated and for some of them, results were already published and will be reviewed. In general, it appears that at least some TKI are able to show a clinically meaningful tumor control in MTC.

S27.4

Nodules and more
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The pathophysiology of thyroid nodules is still widely undefined even though recent data from microarray studies hint towards a distinct group of candidate genes. Furthermore, a better understanding of new mechanisms involved in thyroid growth regulation like TGF-β, BMP and wnt signalling may open new avenues for treatment. Recent data derived from animal models of thyroid carcinomas suggest an action of thyroid hormones despite the loss of TSH receptor. Upon confirmation these findings will change our clinical approach for the treatment of benign and malignant thyroid nodules. Conventionally, cold benign nodules and thyroid cancers were treated with thyroid hormones under the idea that TSH suppression will influence tumour growth. The recent findings suggest rather a direct thyroid hormone mediated effect which may lead to a new pathophysiological basis for the treatment of thyroid nodules[1].
Brabant G TSH suppressive therapy in thyroid carcinoma: what are the targets? *JCEM*, in press.

GH: structure–function relationship – S28

S28.1

Plasticity in the growth hormone axis

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Pituitary growth hormone (GH) is released in a highly pulsatile fashion in response to stimuli from its hypothalamic regulators, GH releasing hormone (GHRH) and somatostatin (SRIF), as well as feedback from peripheral signals. This interplay is complex, and still poorly understood. GHRH is a major factor in controlling pituitary GH synthesis and somatotroph cell number as well as GH secretion, and lack of GHRH or its receptor cause profound somatotroph hypoplasia and dwarfism. Whilst somatostatin can powerfully suppress GH release, chronic lack of SRIF has more subtle effects on GH secretory patterns, without a major effect on growth. Similarly, Ghrelin powerfully stimulates GH release, but the physiological relevance of endogenous Ghrelin for normal GH secretion remains unclear. For efficient generation of GH pulses, both the hypothalamic mechanisms and the pituitary target cells need to be highly coordinated in their secretory activity. This is important since the target tissue responses to GH depend on both the amount and the pattern of GH exposure. Recent imaging studies using transgenic mice with GH or GHRH cells tagged with GFP have shown that the pituitary cell populations are highly dynamic and show a remarkable degree of plasticity, with both cell number and hormone reserves varying in response to demands at different stages of life. Aging reduces the activity in the GH axis, but the mechanisms are not obviously related to failure or reduction in GHRH or GH cells, suggesting that the age related decrease in GH might be reversible. Analysis of other animal models in which hypothalamic or pituitary cells are disrupted in development or post-natally has shed light on mechanisms underlying similar problems in children with pituitary deficits. The cellular plasticity in the adult pituitary implies the presence of progenitor cells, and we have recently identified and grown such cells in culture, and shown that they can give rise to all the pituitary cell types. Such studies extend our understanding of normal and pathological pituitary plasticity underlying GH secretion, and enable us to test some novel potential therapeutic approaches.

S28.2

The contribution of autocrine human growth hormone to neoplasia

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The hGH gene is expressed in epithelial cells of the normal human mammary gland. Increased epithelial expression of the hGH gene is associated with the acquisition of pathological proliferation, and the highest level of hGH gene expression is observed in metastatic mammary carcinoma cells. Autocrine hGH production in human mammary carcinoma cells results in increased cell proliferation and survival associated with alterations in morphology. We have further demonstrated that autocrine production of hGH in immortalized human mammary epithelial cells concomitantly enhances proliferation and offers protection from apoptosis; forming the basis for abnormal mammary acinar morphogenesis, oncogenic transformation and tumor formation *in vivo*. Thus, simple forced expression the hGH gene is sufficient for oncogenic transformation of the immortalized human mammary epithelial cell. Moreover, autocrine production of hGH, in mammary carcinoma cells with epithelial morphology, promotes mesenchymal cellular morphology, increased cell migration and increased metalloprotease (MMP) activity with subsequent acquisition of invasive behavior both *in vitro* and *in vivo*. Autocrine hGH also increases mammary carcinoma VEGF expression resulting in paracrine induced endothelial cell proliferation, survival, migration/invasion, tube formation and increased tumor angiogenesis *in vivo*. In stark contrast to the oncogenic and metastatic potential of autocrine hGH, exogenous hGH neither supports tumor formation nor invasion by human mammary epithelial cells. We have utilized this discrepancy in the oncogenicity of autocrine and exogenous hGH to identify two autocrine hGH regulated genes, trefoil factors 1 and 3 (TFF1/3), that mediate the oncogenic effects of autocrine hGH in human mammary carcinoma cells. We therefore postulate that autocrine hGH functions as a higher order switch, controlling at least some of the genetic elements required for oncogenic transformation and neoplastic progression of the human mammary epithelial cell.

S28.3

Multireceptor ligands in the treatment of pituitary adenomas

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Using the currently available somatostatin receptor (sst) ligands octreotide and lanreotide, that are mainly sst2 agonists, about 60% of patients with acromegaly are adequately controlled. This prompted the development of new drugs targeting other sst subtypes or other receptors that are also expressed on adenomatous cells. BIM 23244 characterized by a high affinity for sst2 and sst5 had been found *in vitro* to allow a stronger inhibition of GH secretion than octreotide in tumors partially responsive to this treatment. The multi-sst ligand pasireotide was also found both *in vitro* and *in vivo* to suppress GH secretion more efficiently than octreotide in some patients. Preliminary data support a potential interest of this new compound in other pituitary adenomas such as corticotroph tumors. Moreover pasireotide was found to have a favorable terminal elimination half-life in humans. Some clinical findings favor a possible additive effect of dopamine D2 agonists with sst agonists in acromegaly. Hybrid drugs combining structural parts of somatostatin and dopamine were developed over the past few years. The first generation hybrid BIM23A387 displayed an improved ability to suppress GH from cultured somatotroph adenomas with an EC50 about 50 times lower than that of octreotide. Among the second generation 'dopastatins' presenting with high sst2 affinity and some sst5 affinity, BIM23A760 displayed the greatest efficacy over all the compounds tested in the same tumors in suppressing GH secretion. This compound also showed a significant inhibition on thymidine incorporation in GH and non functioning pituitary adenomas. These data are in line with the recent demonstration of a functional and structural cooperation of sst2 and D2 dopamine receptors. BIM23A760 was also shown to have a prolonged effect on GH suppression *in vitro* and *in vivo* in an animal model. Depending on receptor expression in each pituitary tumor type and in each particular tumor these novel multiligand drugs may prove more efficient than currently available agonists.

S28.4

Trafficking and function of GHR and the role of GHBP

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GH acts through a cell surface receptor, GHR, which is a member of the type 1 cytokine receptor family. Cytokine receptors have a single trans-membrane domain and dimerisation is required to activate intra-cellular signalling pathways. In common with other cytokine receptors the extra-cellular domain of the GHR is proteolitically cleaved and circulates as a binding protein. Under physiological conditions GH is in part bound in the circulation and the complex with the binding protein is presumed to be biologically inactive and protected from clearance and degradation. Co-administration of binding protein with GH *in vivo* delays GH clearance and augments its anabolic actions. Thus, like many hormonal systems, binding in the circulation provides an inactive circulating reservoir in equilibrium with free active hormones. Free GH is available to bind the cell surface GHR in a 1:2 complex. We have recently generated a fusion of GH with its extra-cellular domain GHR. In bioassays this ligand receptor fusion proved to be an agonist although with 25 fold less activity compared to native GH. However *in vivo*, in hypophysectomised rats, this ligand receptor fusion had 300 fold reduced clearance and proved to be a potent agonist. In conclusion, GHBP provides a natural reservoir of inactive hormone and fusion of GH to GHBP provides a very long-acting potent GH agonist.

Too early-too late: the timing of puberty – S29

S29.1

The systems biology of puberty-searching for hypothalamic gene networks

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The initiation of mammalian puberty requires an increased pulsatile release of gonadotrophin hormone releasing hormone (GnRH) from the hypothalamus. This increase is brought about by changes in transsynaptic and glial–neuronal

communication. Coordination of this regulatory neuronal–glial network likely requires the participation of a multiplicity of genes hierarchically arranged within discrete, but interactive, networks. The identity and structural features of at least one of these hypothalamic networks has been proposed based on results obtained using high throughput, molecular and bioinformatics strategies, in combination with a system biology approach. Although the genes composing this network have diverse cellular functions, they share the common feature of having been earlier identified as involved in tumor suppression/tumor formation. A prominent member of this group is KiSS1, a gene recently shown to be essential for the occurrence of puberty in mice and humans. Cis-regulatory analysis indicated that the network contains five major hubs (*CDP/CUTL1, MAF, p53, YY1, and USF2*) controlling the network at the transcriptional level. These hubs are not only connected to genes encoding proteins required for intracellular signaling, and cell–cell communication, but also with other upper-echelon genes (*OCT2, TTF1, EAP1*) involved in the transcriptional regulation of the pubertal process. The existence of functionally connected genes controlling the pubertal process is consistent with the concept that puberty is under genetic control, and that the genetic underpinnings of both normal and deranged puberty are polygenic rather than specified by a single gene. This and other networks – yet to be identified – may operate within the mammalian hypothalamus to facilitate and integrate cellular and cell–cell communication programs required for the acquisition of female reproductive competence. Supported by NIH grants HD050798, HD25123, MH65438, U54 HD18185, and RR00163.

S29.2

Long acting GnRH analogues in the treatment of central precocious puberty

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GnRH agonists continually stimulate the pituitary gonadotrophs, leading to desensitization and decreases in LH release and, to a lesser extent, FSH release after an initial flare-up. They have transformed the management of precocious puberty since their initial use in early 80's. However, several issues remain unresolved that are pertinent to the indications and use of these medications. Different GnRH agonists are available in various depot forms and their use results in the regression or stabilization of clinical pubertal symptoms. Gonadotropin levels can be monitored but the optimal level of suppression has not been established. The main endpoints considered for GnRH agonist treatments are short term effects on pubertal development (clinical, biological or by ultrasound examination) and long term effects on height. No controlled trial comparing treated and untreated outcome has been performed and the evaluation relies on the comparison between observed and pretreatment predicted outcomes. Height gains of variable magnitude are observed and are associated with several factors. The optimal time to stop treatment has not been established prospectively. Pubertal signs generally reappear a few months after interruption of GnRH agonist treatment. Long-term fertility has not been fully evaluated, but preliminary results are reassuring. Tolerance is considered satisfactory but treatment may be associated with menopause-like symptoms and local tolerance can be a concern. Concerns have been raised about the possible risk of obesity and osteoporosis. Unresolved issues concern the association of GnRH agonists with other medications such as growth hormone and steroids.

S29.3

Estrogen and kisspeptin regulation of GnRH neurons at puberty

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The recent discovery of the importance of kisspeptin-GPR54 signaling to the onset of puberty in humans has generated intense interest in understanding the mechanisms and pathways of kisspeptin-GPR54 signaling at the gonadotropin-releasing hormone (GnRH) neurons. Using transgenic mouse models, electrophysiology and immunocytochemistry we have shown that kisspeptin strongly activates adult GnRH neurons and that these cells receive a direct input from kisspeptin neurons located in the rostral periventricular region of the third ventricle (RP3V). With respect to the initiation of puberty, data indicate that the GnRH neurons become activated by kisspeptin through a two step process; first, the expression of kisspeptin in RP3V neurons increases abruptly just prior to puberty and, second, the response of GnRH neurons to kisspeptin becomes more pronounced. Recent studies examining the mechanism underlying the up-regulation of kisspeptin expression in the RP3V at puberty, suggest a critical role for circulating estrogens in the week prior to puberty onset. Ovariectomy in the second week of life prevents the up-regulation of kisspeptin expression and this is corrected by estrogen replacement. As GnRH neuron activation is required to initiate estrogen synthesis, these results suggest that the early activation of the GnRH neurons occurs independent of kisspeptin. However, once initiated, circulating estrogens activate RP3V kisspeptin neurons that, in turn, strongly excite the GnRH neurons. Intriguingly, data suggest a similar scenario in the adult whereby estrogen activates RP3V kisspeptin neurons to initiate the GnRH surge leading to ovulation. Thus, it seems likely that an important estrogen-kisspeptin-GnRH neuron signalling pathway develops around the time of puberty and that this is used for the activation of GnRH neurons both at puberty onset and ovulation.

S29.4

Human genetics of pubertal onset

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Pubertal onset results from reactivation of the gonadotropic axis by neuroendocrine mechanisms which remain to be defined. Human genetics is one of the approaches to characterize disease mechanisms and therefore novel physiological system. Two different type of phenotype due to abnormal timing of the pubertal onset are described: advance of the pubertal onset also call central precocious puberty and delayed or absence of puberty. The latter is due to gonadotropic deficiency reflecting hypothalamic or pituitary defects. Genetics of both conditions are clearly different. Multifunctional and polygenic models of transmission have been described in central precocious puberty whereas autosomal or X-linked dominant and recessive transmissions have been reported in gonadotropic deficiency. Several genes defects have now been described as a cause of gonadotropic deficiency. These genes were studied as they were natural candidate genes, they were revealed by genome mapping in informative families or by chromosomal analysis of patients with contiguous gene syndrome but also by analogy with the phenotype observed after their invalidation in mice. These genes encode for proteins involved in the normal migration of GnRH neurones from the olfactory placode toward the hypothalamus, for transcription factors involved in the normal pituitary development or for proteins playing a major role in the neuroendocrine regulation of the gonadotropic axis. Mutations in two or more genes may explain the high phenotype expressivity observed in familial cases with gonadotropic deficiency and reveal oligogenic model of transmission. Some of these genes described in gonadotropic deficiency are now interesting candidate genes for the genetic determinism of central precocious puberty.

Clinical highlights – S30

S30.1

Reversible Kallmann syndrome associated with a novel homozygous mutation in the prokineticin receptor-2 gene

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Reversible Kallmann syndrome (KS) is a rare variant of hypogonadotropic hypogonadism (HH) reported in men, in which gonadotropin, testosterone (T) and fertility recover spontaneously following treatment with gonadotropins or T. In a few cases mutations of FGFR1 and KAL1 genes have been found. In this report we describe a subject with a KS carrying a new homozygous mutation of PROK-R2 gene and displaying an apparent reversal of his reproductive condition. The proband, born from first cousins, was a 19-year-old male with absent puberty, testes of 3 ml, and no sense of smell. FSH and LH basal and GNRH stimulated, and T and inhibin B levels were in the prepubertal range. MRI demonstrated absence of olfactory bulb and hypoplasia of sulci. The patient was firstly treated with gonadotropins and afterwards with T enanthate (TE). Persistence of spermatogenesis, fertility and supranormal levels of plasma T under long-term T administration, and normalization of pituitary-gonadal hormones two years off testosterone replacement therapy indicate a reversal of his HH state. Genetic analysis excluded KAL1 and FGFR1 mutation, and disclosed a T/A base substitution at codon 336 of prokineticin receptor 2 gene (Prok-R2), leading to a Val274Asp mutation in the protein. The mother with delayed menarche and normal sense of smell presented heterozygous mutation in Prok-R2 gene, suggesting that the proband inherited the trait from consanguineous parents. This study describes a novel mutation in the

Prok-R2 gene and extends our understanding of the role of PROK2-PROKR2 gene pathway in the regulation of olfactory and GN-RH producing neurons development in humans. Moreover, present finding confirms that HH cannot be considered an irreversible state in the subjects harboring genetic mutations in known genes involved in the control of GnRH neuron migration and function.

S30.2

Contrast-enhanced ultrasound of adrenal mass: differentiation between benign and malignant lesions

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Background
Adrenal masses can be detected by ultrasound with high sensitivity and specificity. However, a differentiation between benign and malignant adrenal masses is presently not possible. Contrast-enhanced ultrasound has been studied intensively with excellent results for the characterization of liver lesions.
The aim of the present study was to evaluate the value of contrast-enhanced ultrasound for the characterization of adrenal mass in a proof of principle study.
Methods
Thirty-five patients with adrenal incidentaloma received an ultrasound of the adrenal mass, including Duplex and Doppler ultrasound, followed by contrast-enhanced ultrasound. The dynamic of contrast-enhancement was analyzed using time-intensity curves. In addition, all patients received CT or MRI and a detailed laboratory testing including hormone profile. In susceptive cases adrenalectomy was performed.
Results
Early arterial contrast-enhancement and rapid wash-out was seen on contrast-enhanced ultrasound in all patients with primary or secondary malignant lesions of the adrenal gland ($n=6$). All primary malignant lesions were confirmed by histology. In 91% (32/35) of examined patients MRT/CT and contrast-enhanced ultrasound were congruent concerning the characterization of adnoma versus non-adenoma. However, in three of these cases all imaging methods suspected non-adenoma, but histology found adrenal adenoma after adrenalectomy. The sensitivity and specificity of contrast-enhanced ultrasound for the diagnosis of malignant adrenal mass was 100% and 79%, respectively.
Discussion
The present proof of principle study shows that contrast-enhanced ultrasound can be used to differentiate between adenoma and non-adenoma as good as CT or MRI and could be a cost-effective method for pre-selection of patients with adrenal incidentaloma.

S30.3

[^{123}I]Iodometomidate as a radiotracer for diagnosis of adrenal tumours and adrenocortical carcinoma

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Subject
Adrenal tumours (AT) are often detected incidentally and represent a variety of differential diagnoses with variable therapeutic consequences. We have recently developed [^{123}I]Iodometomidate ([^{123}I]IMTO), that specifically binds to adrenal CYP11B enzymes as a SPECT tracer for adrenal scintigraphy. This radiotracer is now evaluated in patients with adrenal tumours in an ongoing clinical trial. The study was approved by the ethical committee and patients gave written informed consent.
Methods
Patients (27 adrenocortical carcinomas (ACC), 12 adrenal adenomas (2 Conn adenomas, 3 overt Cushing adenomas, 3 subclinical Cushing adenomas, 4 nonfunctioning adenomas), 1 renal cell carcinoma and 2 metastases) received 185 MBq [^{123}I]Iodometomidate i.v.. Biodistribution and pharmacokinetics of the tracer were studied by planar scintigraphy, SPECT and SPECT/CT over 24 h.
Results
[^{123}I]Iodometomidat proved to be a tracer that specifically accumulates in adrenocortical tissue. Benign adrenal tumours of known adrenocortical origin were excellently visualized in SPECT/CT after 4–6 h with optimal target to background ratios after 24 h p.i. In contrast, adrenal tumours of known non-adrenocortical origin did not show any tracer uptake. Tracer uptake in patients with ACC was heterogeneous. In several patients only in the early phases a moderate tracer uptake was seen in lesions known from CT, particularly in patients with poorly differentiated tumours and patients receiving mitotane. In contrast, hormone producing ACCs exhibited high and lasting tracer uptake.
Conclusion
Due to its highly specific uptake in adrenocortical tissue [^{123}I]IMTO is a promising tracer for noninvasive differentiation of adrenal lesions. In patients with ACC tracer uptake shows interindividual variation dependent on differentiation status and pretreatment. In ACC patients with high tracer uptake radiotherapy with [^{131}I]Iodometomidate may hold therapeutic potential.

S30.4

Evaluation of endothelial function and endothelial nitric oxide synthase intron 4a/b polymorphism in primary hyperparathyroidism

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The aim of this study is to evaluate endothelial function in patients with primary hyperparathyroidism (pHT) during the preoperative hypercalcemic and post-operative normocalcemic periods and to determine whether intron 4a/b polymorphism of eNOS gene influences endothelial function. Forty-one patients with pHT (28 women, 12 men; age: 48.4±11.6 years, serum calcium: 11.4±1.0 mg/dl, parathyroid hormone: 373.3±394.5 pg/ml) who fulfilled the criteria for parathyroid surgery according to 2002 NIH guidelines, were examined preoperatively and reexamined 5.8±1.9 months after parathyroidectomy. All patients were normocalcemic after surgery. Forty-seven healthy subjects (30 women, 17 men, age: 48.03±8.9 years) were served as control group. Early atherosclerotic changes were determined by flow-mediated dilation of brachial artery (FMD). eNOS4a/b polymorphism was detected by polymerase chain reaction. FMD was significantly lower in patients with pHT preoperatively compared with controls (8.48±1.78% vs 19.52±2.26%, $P<0.001$). FMD improved significantly after parathyroidectomy (16.19±2.16%, $P<0.001$ compared with preoperative measurements), but still significantly lower than controls ($P<0.01$). The distribution of eNOS4a/b genotype frequencies were not significantly different between patients and controls. FMD was not significantly different among patients and controls carrying a allele or b/b genotype. Logistic regression analysis showed that presence of hypercalcemia (>10.5 mg/dl) was the only significant independent predictor of lower FMD (<9.4%). eNOS4a/b polymorphism did not enter in this model. Impaired endothelial function in patients with pHT improves after succesfull parathyroid surgery. No compelling data are evident to suggest that eNOS4a/b polymorphism modify the atherosclerotic process in patients with pHT.

S30.5

Increased prevalence of tricuspid regurgitation in patients with prolactinomas chronically treated with cabergoline: dose and treatment duration effect

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Background
Cabergoline, a dopamine receptor-2 agonist, used to treat prolactinomas was associated with increased risk of cardiac valve disease.

Objective
To evaluate prevalence of cardiac valve regurgitation in patients with prolactinomas treated with cabergoline.
Design
Open, case–control, prospective.
Patients
Fifty patients (44 women, 6 men) and 50 sex- and age-matched control subjects.
Intervention
In the patients last cabergoline dose was 0.5–7.0 mg/week (1.3 ± 1.3 mg/week): $<$ 1 mg/week in 44%, 1–3 mg/week in 46% and >3 mg/week in 10%. Treatment duration was 12–60 months in 32% and >60 months in 68%. The cumulative (mg $\times$ months of treatment) dose of cabergoline ranged 32–1938 mg (median 280 mg).
Measurements
Valve regurgitation was assessed according to the recommendations of the American Society of Echocardiography.
Results
Prevalence of mild regurgitation of mitral (22% vs 12%, $P=0.29$), aortic (4% vs 2%, $P=1$), tricuspid (30% vs 42%, $P=0.29$) or pulmonic (12% vs 6%, $P=0.48$) valves was similar in patients and controls while moderate tricuspid regurgitation was higher in the patients (54% vs 18%, $P<0.001$). Moderate tricuspid regurgitation was more frequent in patients receiving a cumulative dose above the median (36% vs 72%, $P=0.023$) than in dose receiving a lower dose, who had a prevalence similar to controls (36% vs 18%, $P=0.15$).
Conclusion
Moderate tricuspid regurgitation is more frequent in patients taking cabergoline than in control subjects. Mostly in patients treated at higher cumulative doses. A complete echocardiographic assessment is indicated in patients treated long-term with cabergoline, particularly in those requiring elevated doses.

S30.6

Association of pioglitazone treatment with decreased bone mineral density in obese premenopausal patients with polycystic ovary syndrome: a randomized, placebo-controlled trial

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Background
PPARγ-agonist treatment may decrease bone mineral density (BMD) in postmenopausal women. The hypothesis that premenopausal women are relatively protected from mineral loss during PPARγ-agonist treatment has not been evaluated in clinical studies.
Aim
To investigate the effect of pioglitazone on BMD and bone turnover markers in patients with polycystic ovary syndrome (PCOS).
Methods
Thirty premenopausal PCOS patients were randomized to 16 weeks of pioglitazone (30 mg/day) or placebo treatment. Patients underwent measurements of BMD (hip (neck, total), lumbar spine (L2–L4)), bone metabolic parameters (alkaline phosphatase (ALP), 25-hydroxyvitamin D (25OHD), C-telopeptide of type I collagen (ICTP), osteocalcin, and parathyroid hormone (PTH)), endocrine profiles (testosterone, estradiol, and insulin), and measurements of body composition (WHR, BMI, and whole body DXA scans). Fourteen age and weight-matched females were included as a control group.
Results
PCOS patients had significantly higher levels of ICTP, fasting insulin, and testosterone than controls, whereas no differences were measured in ALP, PTH, body composition, or BMD.
Pioglitazone was followed by reduced BMD: Lumbar spine 1.140 (0.964–1.348) vs 1.127 (0.948–1.341) g/cm^2 (average decline 1.1%) and femoral neck 0.966 (0.767–1.217) vs 0.952 (0.760–1.192) g/cm^2 (average decline 1.4%), both $P<0.05$. ALP and PTH significantly decreased during pioglitazone whereas no significant changes were measured in 25OHD, ICTP, osteocalcin, estradiol, testosterone, and body composition.
Conclusion
Pioglitazone treatment was followed by decreased lumbar and hip BMD and decreased measures of bone turnover in a premenopausal study population relatively protected against bone mineral loss.

Meet the Expert Sessions

ME1

The impact of abdominal obesity

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Overnutrition, increased macronutritient intake, physical inactivity, and ageing are associated with expansion of adipose tissue mass and cytokines, favoring in genetically and metabolically susceptible subjects, the development of insulin resistance, metabolic syndrome and diabetes. Adipose tissue distribution in human is dependent on genetic and environmental factors. The control of the rate of filling of adipocytes seems to be the main factor determining the local, regional mass of adipose tissue. Causes of visceral fat accumulation include glucocorticoid excess or decreased estrogen/androgen ratio either in plasma or within adipose tissue. Intra-adipose sex steroid metabolism is a determinant of gynoid versus androgen patterns of body fat. Abdominal obesity is associated with greater risk for hypertension, dyslipidemia, type 2 diabetes and coronary heart disease, due to increased release of free fatty acids from visceral fat to the liver. Visceral fat is highly active metabolic and endocrine organ that secretes many adipokines with action at local and systemic level. Dysregulation of adipokines contributes to the pathogenesis of the obesity-associated metabolic syndrome, resulting in insulin resistance, type 2 diabetes, hypertension, hyperlipidemia and vascular disease. Sex differences in visceral fat lipolysis are responsible for more cardiovascular complications in men than in women. Obesity, insulin resistance and type 2 diabetes are characterized by chronic low-grade inflammation. Adipose tissue of obese insulin resistant subjects is characterized by increased expression and/or secretion of inflammatory molecules, including TNF-a, IL-6, PAI-1 and leptin while the insulin-sensitizing factor adiponectin is downregulated. Macrophage infiltration in adipose tissue in obesity contribute to the reported inflammatory profile in abdominal obesity. Local inflammation in the expanded adipose tissue mass among obese subjects is proposed to be a partly responsible for obesity-related insulin resistance. Visceral obesity might also be a marker of defective fat portioning between the adipose tissue, the skeletal muscle, the liver and the heart.

ME2

Rational for using insulin analogues in the treatment of diabetes mellitus

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In both Type 1 and Type 2 diabetes mellitus (T1, T2DM) there is stringent need to maintain A1C $<$ 7.0% since clinical diagnosis all life long. Large, prospective, intervention trials have proven that A1C $<$ 7.0% is the most (and the only!) effective mean to prevent onset of vascular complications of diabetes and/or delay its progression.

In T1DM, it is mandatory to use basal + meal-time insulin. The gold standard is the continuous s.c. insulin infusion (CSII) with rapid-acting insulin analogues. As compared to multiple daily injections (MDI) based on rapid- and long-acting insulin analogues, CSII provides the highest flexibility in life-style, although A1C and risk of hypoglycaemia are no superior vs MDI. With MDI, the basal insulin need should be replaced with a long-acting analogue, either glargine once daily, or detemir twice daily (in the majority of subjects). NPH should no longer be in use in T1DM (including children and elderly people) because of the risk for nocturnal hypoglycaemia and hypoglycaemia unawareness. Rapid-acting analogues (lispro, or aspart or glulisine), not human regular insulin, should be given at each meal, including snacks in children, adults and elderly people with T1DM. The dual advantage of rapid-acting analogues vs human regular insulin, is lower post-prandial hypoglycaemia and lower A1C, with less risk for hypoglycaemia and greater flexibility (improved life-style).

In T2DM, the present recommendation is aggressive treatment of hyperglycaemia to lower A1C to below 7.0%, initially with life-style changes + metformin, but immediately after with early initiation of basal insulin and/or additional oral drugs whenever A1C is not at target within three months (ADA/EASD consensus, 2006). Insulin in T2DM should be initiated as evening administration of either the cheap NPH insulin, or the more sophisticated (and expensive) long-acting insulin analogues glargine (once daily), or detemir (once or twice daily in more than 50% of T2DM subjects). All basal insulins NPH, glargine and detemir keep A1C $<$ 7.0% in T2DM failing to oral drugs, but the risk of hypoglycaemia is about 50% less using glargine or detemir versus NPH. Because prevention of hypoglycaemia is at least as important as it is reduction in A1C, the basal insulin of choice is a long-acting insulin analogue. When starting basal insulin in T2DM, it is important that the evening insulin dose is titrated to fasting normoglycaemia (100 mg/dl, 5.5 mmol/l). The titration may require weeks of time and large dose in subjects who are insulin resistant due to obesity, primarily visceral obesity (fatty liver). The modern view is that a large insulin dose should not worry neither the doctor not the patient because insulin is healthy. When meal-time insulin is needed, rapid-acting insulin analogues (lispro, or aspart or glulisine) should be used in T2DM, not human regular insulin.

ME3

Contraception in the new millennium

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The control of fertility constitutes a global health issue, because overpopulation and unintended pregnancy have both major personal and societal impact. Oral contraceptives (OCs) have been the gold standard for contraception since their introduction in 1960. They are used for both their contraceptive and noncontraceptive benefits. Hormonal contraceptives are made of either oestrogen-progestin combinations or progestins alone. Attempts have been made to use other classes of steroids for contraception such as the so-called selective progesterone receptor modulators (SPRM). Hormonal contraceptives can be employed through different routes (intramuscularly, intranasally, intrauterus, intravaginally, orally, subcutaneously, and transdermally). The newest developments in contraception include low and ultra-low doses of estrogen, the use of less-androgenic 19 nor-testosterone progestins, and the nonsteroidal progestin drospirenone, a new minipill (progestin-only preparation) containing desogestrel, the contraceptive transdermal patch, the vaginal estrogen-progestin ring, the levonorgestrel intrauterine system and several subcutaneously implanted systems (contraceptive 'rods' and 'capsules'). Although the traditional dosing regimen, 21 active pills and 7 placebo pills, (21/7), reduces many symptoms women suffer with spontaneous cycles and hormone withdrawal symptoms. New contraceptives are available that increase the time between hormone-free intervals. Extended cycle contraception is a safe and acceptable form of contraception and may be more efficacious than cyclic regimens. Most extended cycle regimens result in fewer scheduled bleeding episodes, as well as in fewer problems with bloating, menstrual symptoms and dysmenorrhea. Women usually experience more unscheduled spotting and bleeding in the initial cycles, but those problems decrease with longer use. Some women have medical conditions that make extended cycle contraception a preferred method. Counseling women about all their contraceptive options and the variety of ways that OCs can be taken may increase women's commitment on the selected therapy. Finally, the recently observed enhancement of our knowledge related to the basic processes of reproduction, as well as the genomic and proteomic revolutions provide new targets for contraceptive development.

ME4

Two-photon microscopy of cancer invasion and metastasis

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Multiphoton microscopy has defined standards for 3D fluorescence and higher harmonic generation analysis of cells and tissue structures *in vitro* and *in vivo*. Compared to single-photon excited confocal microscopy, two-photon microscopy utilizes near-infrared (NIR) excitation generating twice to multi-fold enhanced tissue penetration, reduced light scattering and minimized phototoxicity and photobleaching at out-of-focus regions, yet preserves submicron spatial resolution and subcellular detail of cell and tissue structures. Using invasive tumor xenografts in the dorsal skin-fold chamber in nude mice, we here show the dynamics of tumor growth, neoangiogenesis, and tumor invasion into the adjacent tissue microenvironment. Using fluorescent labels, not only single cells but also extensively invading collective cell strands were reconstructed to move along and around preexisting blood and lymphatic vessels, not however neovessels. Using dual-color cells expressing Histone-H2B/eGFP in the nucleus and cytoplasmic RFP, the combined dynamics of collective invasion and mitotic activity defines the hallmarks of 'invasive growth'. *In vivo* imaging now allows to study molecular programs controlling metastatic cancer progression are diverse in different cancers as well as within the microenvironment of a single lesion. These include amoeboid, mesenchymal and collective invasion processes as well as cellular and molecular plasticity during molecular intervention. In future studies, time-resolved two-photon microscopy will allow to gain novel insight into the mechanisms cancer progression, regression, and persistence during experimental therapy.

ME5

How valid is a biochemical diagnosis of chromaffin tumors?

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Phaeochromocytoma (PH) and functional paraganglioma (FPGL) are neoplasm of chromaffin tissue that synthesise catecholamines and are mostly located in adrenal medulla (PH) or elsewhere (FPGL). Patients may also harbour non-secreting head and neck paragangliomas (PGL). Up to ¼ of PH/PGL are familial. A high degree of suspicion for PH and FPGL should be risen in case of spells, resistant hypertension, family history of PH/PGL, a genetic syndrome that predispose to PH (e.g. MEN2), a past history of resected PH and present history of recurrent hypertension and spells, and an incidentaloma with radiologic finding consistant with PH. The laboratory diagnosis of PH and FPGL relies on the identification of excessive secretion of catecholamines and/or their derivatives, which include norepinephrine (NE), epinephrine (E), and rarely dopamine (D) and their methoxylated metabolites metanephrines, such as normetanephrine (NM), metanephrine (M) and 3-methoxytyramine (3Met). These metabolites are produced continuosly and independently of catecholamine release, which may be modest, absent or paroxysmal, therefore should provide more accurate tests to diagnose PH. Consequently, current recommendations state that initial biochemical testing should include measurement of either plasma or urinary fractionated metanephrines or both. Metanephrines are further metabolised to sulphate conjugates resulting in concentrations up to 25 times the corresponding free compounds. Gastrointestinal tissues and renal function may influence the levels of conjugated metabolites. In urine, the common assay measures the sum of free and conjugated metanephines, while in the plasma they are determined separately. This might explain the diagnostic advantage of plasma compared to urinary measurement reported by some A. However, the small advantage of plasma free metanephrines assay in term of sensitivity does not likely account for its technical difficulty, and the reported differences between the performance of the 2 methods are small compared to the advantages of either test to tests for the parent plasma and urinary fractionated catecholamines. Sensitivity and specificity of these 4 assays will be further discussed, as well of VMA. In general, high sensitivity is associated with trade-off in specificity, with difficulties in distinguishing TP from FP. The extent of elevation should be considered. Any role for chromogranin A, NPY, NSE?

ME6

Beyond the bone: sporadic and hereditary hyperparathyroidism

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The past 15 years resulted in a great progress of our understanding regarding the genetic basis, pathogenesis, symptomatology, laboratory diagnosis and differential diagnosis of hypercalcemic disorders both of those occurring seemingly sporadically and of those developing in a familial setting. Since serum calcium measurement takes as part of the routine laboratory screening examinations the number of asymptomatic and also of familial cases of primary hyperparathyroidism are increasing worldwide.

This session will review the most important new knowledge about sporadic primary hyperparathyroidism (PHPT) as well as inherited parathyroid-dependent hypercalcemic syndromes, including the genetic diseases of the calcium-sensing receptor (CaSR).

Nowadays, PHPT is the third most common endocrine disorder after diabetes mellitus and thyroid diseases. Its prevalence is 3–4 per 1000 in northern Europe. Among elderly patients (≧75 years) the prevalence could be as high as 21 per 1000. According to some estimation, 80% of PHPT cases are asymptomatic, while the familial forms represent about 5% of all cases. There are four autosomal dominant hereditary syndromes frequently associating with PHPT: multiple endocrine neoplasia type 1 (MEN1), multiple endocrine neoplasia type 2 (MEN2), hyperparathyroidism–jaw tumor syndrome (HPT-JT) and familial isolated hyperparathyroidism (FIHPT). Until the cloning and sequencing of the putative FIHPT gene(s), the diagnosis of FIHPT rests on the exclusion of other specific endocrine and non-endocrine tumors featuring MEN1, MEN2 and HPT-JT syndromes, as well as CaSR mutations.

The hypercalcemia of familial hypocalciuric hypercalcemia (FHH) is usually mild (<3.0 mmol/l) and asymptomatic, and it associates almost always with low calcium clearance to creatinine clearance ratio. The relative frequency of FHH among patients with PTH-dependent hypercalcemia is not exactly known, it was estimated to be about 7%.

ME7

The sequelae of brain injury: is the pituitary involved?

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Traumatic brain injury (TBI) which is a worldwide public health problem has been recently recognized as a cause of neuroendocrine dysfunction. Pituitary dysfunction due to TBI may be partial or complete and in retrospective studies as many as 25–50% of patients have been demonstrated to have some degree of pituitary hormone deficiencies. Recently five prospective studies investigating the 12 months follow-up of anterior pituitary function after TBI have been published. Based on these and one recently published 3 years prospective study, pituitary dysfunction in brain-injured patients may improve over time or although rare, may also worsen. Similar to TBI, aneurysmal subarachnoid haemorrhage was also demonstrated as a risk factor for pituitary dysfunction.

Another kind of brain injury is chronic repetitive trauma which is seen in combative sports. Concussion, a common lesion after TBI, is an injury associated with sports including boxing and kickboxing. Until recently, there was no study reporting pituitary dysfunction due to sports induced head trauma. In a preliminary study, we have investigated pituitary functions in amateur boxers for the first time and GH deficiency was very common among retired amateur boxers. In another study, amateur kickboxing is demonstrated to be a novel cause of hypopituitarism and kickboxers are found to be at risk for hypopituitarism.

Little is known regarding the pathogenesis of brain injury induced hypopituitarism due to various causes. Interesting novel studies that will highlight this field will be discussed.

ME8

microRNA

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MicroRNAs (miRNA) are small (18–24 nts) non-coding RNAs which are part of an evolutionarily highly conserved intracellular mechanism to regulate gene expression in a sequence-specific manner. These regulatory RNAs function by acting as sequence-specific guides which recruit multi-protein complexes to target mRNA sequences which are subsequently silenced. miRNAs are processed from primary transcripts (pri-miRNA) by cellular components which are also at least partially involved in the process of RNA interference (RNAi). Various functions have been attributed to miRNAs including regulation of cellular proliferation, differentiation, and apoptosis. Moreover, aberrant expression of specific miRNAs has recently been described in human lymphoma and leukemia. In particular, BCR-ABL and c-MYC dependent over-expression of the polycistronic and oncogenic miR-17-92 cluster (encoding miR-17, miR-18a, miR-19a, miR-20a, miR-19b, and miR-92) has been described in chronic myeloid leukemia (CML) cell lines, primary CD34+ cells from CML patients, and in lung cancer. Moreover, in BCR-ABL positive K562 cells, miR-17-92 encoded miRNAs repress luciferase activity in miRNA-specific reporter assays. In addition, lentivirus-mediated over-expression of miR-17-92 increases both cell proliferation and sensitivity to imatinib induced cell death. So far, the function of and the targets regulated by individual miRNAs, in particular of those encoded on polycistronic transcripts, are largely unknown. Finally, strategies to induce stable gain- and loss-of-function phenotypes for specific miRNAs based on lentiviral transfer of miRNA- and antagomir expression cassettes will allow functional analysis of individual miRNAs. Such studies are required to determine whether altered miRNA expression may contribute to the pathophysiology of CML and how miRNAs may provide potential targets for therapeutic intervention.

ME9

Prolactin: an underestimated hormone

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Prolactin (PRL) is a peptide hormone and secreted by the lactotrophs in the anterior pituitary gland. Its secretion is regulated by the hypothalamus and under control of inhibitory effect of mainly dopamin. Gamma-aminobutyric acid (GABA) and other unidentified prolactin-release-inhibiting factors has also effects on PRL secretion. Thyrotropin-releasing hormone (TRH) is a prolactin-releasing factor. Vasoactive intestinal peptide (VIP), oxytocin and galanin are the other probable prolactin-releasing-factors.

Human PRL is a single-chain polypeptide of 199 amino acids. It has a molecular weight of 23 kDa but several variants of PRL exist.
Besides its main lactogenic action it is now known that, from animal studies, has several other actions including osmoregulation, reproduction, behaviour modification and immune modulation. In animal models tumour-promoting role of locally produced PRL PRL in breast and prostate has been established. Local production of PRL in breast and prostate tissues in human has also been demonstrated but its role in tumourogenesis is not known. It has been suggested that increased PRL level is a risk factor for human breast and probably prostate cancer but more data is needed to prove this.
PRL is proposed as a metabolic hormone as well. PRL is locally secreted from adipose tissue. PRL excess results in increased food intake and body weight in animal models. Recent data indicate that PRL has a role in insulin sensitivity. PRL stimulates insulin release and regulates adipokine secretion. A role of prolactin in obesity related complications has also been suggested.
Immunoregulatory effects of PRL have also been extensively studied. PRL is secreted by lymphocytes. There is evidence that PRL has acute and chronic effects on immune and autoimmune responses. In animal models of rheumatoid arthritis, systemic lupus erythematosus, multiple sclerosis and uveitis suppression of prolactin by bromocriptine improves disease outcome. There are several studies in which bromocriptine has been demonstrated to suppress autoimmune responses in rheumatic and autoimmune diseases.
Hyperprolactinemia refers to an increase in circulating levels of PRL and is the most common pituitary hormonal abnormality. There are several causes of hyperprolactinemia. Hypothalamic lesions such as tumours and inflammatory processes and drugs such as alpha-methyldopa diminishes dopamin secretion and may cause hyperprolactinemia. Lesions of the pituitary stalk, such as stalk tumors and inflammation, may also cause hyperprolactinemia due to impaired dopamin transport to lactotrophs. Drugs that act as dopamin-receptor-blocking agents such as chlorpromazine, haloperidol, metoclopramide, sulpiride and domperidon block the effects of dopamin and give rise to hyperprolactinemia. Hypothyroidism, estrogens, chest wall lesions are other causes of hyperprolactinemia. Prolactinomas are the PRL secreting tumours of the pituitary gland and cause hyperprolactinemia. These are the most common type of hormone secreting pituitary tumors and the most common cause of tumoral hyperprolactinemia.
Clinical manifestations of hyperprolactinemia are galactorrhea and hypogonadism. Signs and symptoms of the causing disorder and manifestaions of other hormonal dysregulations may also be found in patients. Menstruel abnormalities-amenorrhea or oligomenorrhea- are usually seen in hyperprolactinemic women. Infertility is another manifestation in both genders. Decrease in bone mineral dansity as a result of estrogen deficiency can also be seen. Puberty may be delayed in adolescents. Gynaecomasty and galactorrhea are rarely seen in men.
Differential diagnosis of hyperprolactinemia is very important for clinicians. The most common reasons in clinical practice are pharmacotherapeutic agents. It should be kept in mind that mild stress, even the stress of venepuncture, can induce transient elevations in serum PRL. Initial assessment of a patient with hyperprolactinemia should include medical and drug history and careful physical examination. Serum prolactin levels are ideally measured in the morning in a fasting state. Prolactin levels should not be measured after an examination since the stress of a gynaecological or breast examination may raise the prolactin levels. Biochemical assessment should include beta-hCG, renal and liver function tests, thyroid functions. If serum PRL level is mildly elevated the test should be repeated before further evaluation. Since hyperprolactinemia can be seen in patients with PCOD this should be ruled out in patients presenting with oligo-amenorrhoea. PRL may form immune complexes and may produce 'macroprolactin'. Since this molecule is biologically inactive but can be detected by the PRL assays, this possibility should be kept in mind especially if the patient has no apparent hyperprolactinemic symptoms. Polyethylene glycol precipitation is the method of choice to confirm macroprolactinaemia. In patients with clinical signs and symptoms of hyperprolactinaemia and normal serum prolactin levels, the high-dose hook effect needs to be considered.
Magnetic resonance (MR) imaging of the sellar region should be performed in patients with persistently elevated PRL levels.
The objectives of treatment of hyperprolactinaemia are to normalize serum PRL levels and resolve clinical manifestations. Indications for treatment are: macroprolactinoma, patients with menstrual irregularities, infertility, tumoural compression symptoms and low estrogen levels. Treatment options are pharmacotherapy, surgery and irradiation. Asymptomatic patients may not be treated but should be observed periodically.

ME10

How to prevent osteoporosis?
Marek Bolanowski
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Osteoporosis is the most common metabolic bone disease. It represents significant burden due to fractures and costs for the health system and individuals. There are several factors which increase the risk of osteoporosis, genetic, hormonal and environmental influences are the most important. The primary goal of early intervention is to prevent the fractures. This could be carried out by the influence on bone density and quality, normalization of bone turnover, regulation of hormonal disorders and decrease of environmental harmful effects. The latter are smoking, excessive alcohol and caffeine intake and medication influencing either bone metabolism or predisposing to falls. For the attaining optimal peak bone mass, except for the genetic factors, normal-time puberty, nutrition and activity is necessary. Later in women's life in their reproductive age, regular bleeding and maintenance of normal body mass together with life-style factors are important. Since the majority of osteoporotic fractures follow falls, the falling prevention is of great impact. Among the factors of significant role for the prevention of osteoporosis, the preservation of regular menstrual bleeding, appropriate calcium and vitamin D supplementation and adequate physical activity should be advised. They must be based on the family and individual medical history, presence of risk factors and individually tailored to the subject.

ME11

More than a change of nomenclature: disorders of sex development (DSD)
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Disorders of sex development (DSD) include a heterogeneous group of heritable disorders of sex determination and differentiation, formerly termed 'intersexuality'. This includes chromosomal as well as monogenic disorders, which inhibit or change primarily genetic or endocrine pathways of normal sex development. However, in most patients affected, no definitive cause for the disorder can be found. Therefore, the birth of a child with ambiguous genitalia still represents an enormous challenge. For the structuring of diagnostic procedures, for decision making and also for therapeutic interventions a highly specialized team of physicians of different subspecialties and of experts for psychosocial care is needed to counsel parents and patients accordingly. This meet-the-expert session will focus on the genetic and molecular origins of DSD, the new DSD nomenclature, the consecutive classification and steps for diagnosis. Cases of DSD with special challenges will be presented and discussed with the auditorium.

ME12

Metabolomics and metabonomics
Matej Oresic
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Metabolomics is a discipline dedicated to the global study of small molecules (i.e. metabolites), their dynamics, composition, interactions, and responses to interventions or to changes in their environment, in cells, tissues, and biofluids. Metabolites are the end products of cellular regulatory processes, and their levels can be regarded as the ultimate and amplified response of biological systems to genetic or environmental changes. However, only in the past years, technologies have been developed that allow comprehensive and quantitative investigation of a multitude of different metabolites. The maturation of metabolomics technologies is expected to have profound effect on biomedical research.
We will describe the metabolomics technology and methods, along with selected applications in the context of lipid disorders, obesity and diabetes.

ME13

Endocrine management of gonadal tissue conservation
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Advances in the diagnosis and treatment of childhood, adolescent and adult cancer have greatly increased the life expectancy of young women with cancer, but have resulted in a growing population of adolescent and adult long-term survivors of childhood malignancies, who may experience premature ovarian failure (POF) and infertility as a result of aggressive chemotherapy and radiotherapy treatments (indicated for both cancer and bone marrow transplantation (BMT)).

Ovaries are very sensitive to cytotoxic treatment, especially to radiation and alkylating agents, which are classified as high risk for gonadal dysfunction. The type and dose of chemotherapeutic agent are known to influence the progression to ovarian failure, with alkylating agents increasing the risk of POF by a factor of 9. Cyclophosphamide is the agent most commonly implicated in causing damage to oocytes and granulosa cells in a dose-dependent manner. This follicular destruction generally results in the loss of both endocrine and reproductive functions, depending on the dose and the age of the patient. Indeed, Larsen *et al.* reported a 4-fold increased risk of POF in teenagers treated for cancer, rising to 27-fold in women between 21 and 25 years of age.

Several options are currently available to preserve fertility in cancer patients and allow them to conceive when they have overcome their disease: embryo cryopreservation, oocyte cryopreservation and ovarian tissue cryopreservation. The choice of the most suitable strategy depends on different parameters: the type and timing of chemotherapy, the type of cancer, the patient's age and partner status.

The only established method of fertility preservation is embryo cryopreservation, according to the Ethics Committee of the American Society for Reproductive Medicine, but this option requires the patient to be of pubertal age, have a partner or use donor sperm, and be able to undergo a cycle of ovarian stimulation, which is not possible when chemotherapy has to be initiated immediately or when stimulation is contraindicated according to the type of cancer.

Cryopreservation of oocytes can be performed in single women who are able to undergo a stimulation cycle, although the effectiveness of this technique is still low, with pregnancy and delivery rates ranging from 1 to 5% per frozen oocyte.

Cryopreservation of ovarian tissue is the only option available for prepubertal girls, and for woman who cannot delay the start of chemotherapy. Ovarian tissue can theoretically be frozen using three different approaches: as fragments of ovarian cortex, as entire ovary with its vascular pedicle or as isolated follicles. The indications for cryopreservation of ovarian tissue in case of malignant and non-malignant disease are summarized in a recent review. For patients who need immediate chemotherapy, ovarian tissue cryopreservation is the only possible alternative.

The main aim of this strategy is to reimplant ovarian cortical tissue into the pelvic cavity (orthotopic site) or a heterotopic site like the forearm or abdominal wall once treatment is completed and the patient is disease-free.

To date, we have performed 8 reimplantations of cryopreserved ovarian cortex in 6 women, 2 of them undergoing reimplantation twice.

ME14

Management of GI-NET

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According to the WHO classification system the majority of endocrine tumours of the gastrointestinal tract (GI-NETs) are considered as being well differentiated benign or malignant tumours. Almost all GI-NETs are derived from enterochromaffin or Kulchitsky cells that can synthesize, store and secrete serotonin. The majority of these tumours are non-functioning and their presentation is non-specific. Functioning tumours, may exhibit protean clinical presentation, depending on the combination of bioactive substances (serotonin, tachykinins, kallikreins, prostaglandins among others) that they secrete. The typical 'carcinoid syndrome' occurs in less than 10% of patients and is clinically manifested as cutaneous flushing and gut hypermobility with diarrhoea. Cutaneous flushing of the face, neck and upper chest are characteristic features; less common manifestations include cardiac valvular abnormalities, abdominal pain, and bronchospasm. Symptoms can occur spontaneously or triggered by alcohol intake, serotonin-rich foods and exercise. Carcinoid crisis is an extreme and life threatening expression of the carcinoid syndrome occurring as a consequence of the massive release of amines into the circulation following anaesthesia, interventional procedures or medication. Hypotension, rarely hypertension, tachycardia, bronchial wheezing and central nervous system dysfunction are the main features. Rarely, an atypical carcinoid syndrome or syndromes related to other biologically active substances released by the tumours may occur and dominate the clinical picture. In the majority of patients the carcinoid syndrome can be treated with somatostatin analogues, interferon alpha or combinations. Severe carcinoid heart valve disease is treated with valve replacement. Additional therapies are surgery, surgical debulking, radiotherapy, liver dearterialization, liver (chemo- or radio) embolization, alcohol sclerotherapy of liver metastases, radiofrequency ablation of liver metastases, cryosurgery of liver metastases, occasionally liver transplantation, radiotherapy-coupled somatostatin analogues, 1312I-MIBG and occasionally chemotherapy.

ME15

Histopathology of thyroid tumors

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The most frequent problems of the morphological diagnosis of thyroid tumors with clinical relevance will be discussed under four major headings:

(a) Differential diagnosis of follicular patterned lesions: follicular adenoma (encompassing adenomatous lesions of multinodular goitre), follicular carcinoma and follicular variant of papillary carcinoma. In this setting we will discuss the role of cytology (nuclear features), immunohistochemistry (Ki67/MIB1, CK19, Galectin3, HBME-1, histo-blood group antigens) and molecular genetics (RAS mutations, PAX8-PPARg rearrangement).

(b) Identification of the subgroups of follicular carcinoma: minimally invasive, widely invasive and, most important, though frequently forgotten, angioinvasive. Additional points: Is there room for the evaluation of the degree of vascular invasiveness? And for the presence of Hürthle cells?

(c) Identification (and clinical significance) of the variants of papillary carcinoma (PTC) emphasising some issues: Is there anything one can call '*in situ*' transformation towards PTC within a benign nodule? Microcarcinoma or microtumor? What to do with the encapsulated PTC? What about the Hürthle cell (oncocytic) variant of PTC? Does molecular genetics help in the cytological diagnosis of PTC?

(d) Diagnosis of poorly differentiated carcinoma (PDTC) following the criteria of the Turin Consensus Meeting. In this field it is crucial to distinguish PDTC from the solid variant of PTC and from anaplastic (undifferentiated) carcinoma, taking into consideration morphology (e.g. nuclear features, mitoses, necrosis, invasiveness), immunohistochemistry (thyroglobulin, p53) and molecular genetics (there are a couple of promising therapeutic targets).

In case there is free time at the end of the Session we will discuss some practical (and old) issues such as the limitations of cytopathology and the strategy for studying multinodular goitres, as well as some recent developments in the field of familial thyroid tumors (eg. mutations in GRIM19 and SDH genes).

Selected references

1. Fonseca E *et al.* Diagnostic criteria in well-differentiated thyroid carcinomas. *Endocr Pathol* 2006 **17** 109–117.
2. Lima J *et al.* Germline succinate dehydrogenase subunit D mutation segregating with familial non-RET C cell hyperplasia. *J Clin Endocrinol Metab* 2003 **88** 4932–4937.
3. Lloyd RV *et al.* Observer variation in the diagnosis of follicular variant of papillary thyroid carcinoma. *Am J Surg Pathol* 2004 **28** 1336–1440.
4. Máximo V *et al.* Somatic and germline mutation in GRIM-19, a dual function gene involved in mitochondrial metabolism and cell death, is linked to mitochondrion-rich (Hürthle cell) tumours of the thyroid. *Br J Cancer* 2005 **92** 1892–1898.
5. Rosai J *et al.* Renaming papillary microcarcinoma of the thyroid gland: the Porto proposal. *Int J Surg Pathol* 2003 **11** 249–251.
6. Sobrinho-Simoes M *et al.* Hurthle (oncocytic) cell tumors of thyroid: etiopathogenesis, diagnosis and clinical significance. *Int J Surg Pathol* 2005 **13** 29–35.
7. Sobrinho-Simoes M *et al.* Molecular pathology of well-differentiated thyroid carcinomas. *Virchows Arch* 2005 **447** 787–793.
8. Trovisco V *et al.* BRAF mutations are associated with some histological types of papillary thyroid carcinoma. *J Pathol* 2004 **202** 247–251.
9. Volante M *et al.* Poorly differentiated thyroid carcinoma: the Turin proposal for the use of uniform diagnostic criteria and an algorithmic diagnostic approach. *Am J Surg Pathol* 2007 **31** 1256–1264.

ME16

HRT in women: a never ending story?

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The history of hormone replacement therapy (HRT) has been marked out by successive 'earthquakes', the perception of which has been reflected by the evolution of prescription curves. Early hormonal treatment protocols included estrogens only and have lead to an increased relative risk of endometrial cancer. This risk returns to baseline if a progestin is associated to estrogens for at least 12 days/28.Then based on interventional studies as well as *in vitro* data, animal studies, and surrogate markers suggesting a protective effect of HRT on vascular disease, HRT has been widely

Oral Communications

Neuroendocrinology and pituitary – OC1

OC1.1

Structured assessment of neuroendocrine dysfunction following traumatic brain injury and aneurysmal subarachnoid hemorrhage in 921 patients: the German Interdisciplinary Database

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Background
Recent studies show that traumatic brain injury (TBI) and aneurysmal subarachnoid hemorrhage (SAH) are frequent causes of long-term disturbances of hypothalamo–pituitary function. Still little is known about risk factors and clinical characteristics of pituitary impairment after brain damage. This study aimed to address these questions on a larger scale by establishing a national registry of these patients.
Methods
All centers treating patients with TBI or SAH and performing endocrine assessments can include patients. Data were collected using a structured, internet-based study sheet, obtaining information on clinical, radiological and hormonal parameters.
Results
To date, 921 patients (594 TBI, age 43.5 ± 19.7 years; 324 SAH, 49.7 ± 11.8 years) have been included. Stimulation tests for the corticotropic and somatotropic axes were performed in 241 (26%) and 206 (22%) patients, respectively. Information of pituitary function was still lacking in many patients. In patients with known pituitary function, hypopituitarism was reported in 44 and 29% after TBI and SAH, respectively. When we considered only patients with at least one stimulation test for the corticotropic and somatotropic axes, the frequencies of hypopituitarism after TBI and SAH were 52 and 65%, respectively. According to the frequency of impariment, pituitary hormone secretion was impaired the following sequence: ACTH, GH, LH/FSH, and TSH.
Conclusions
Our data confirm that hypopituitarism is a common complication of TBI and SAH. We cannot exclude a certain selection bias for performing endocrine stimulation tests only in more severely affected subjects. Nevertheless, the fact that pituitary impairments are remarkably more common in patients with than without endocrine stimulation tests implies that hypopituitarism might be overlooked if only basal values are performed.

OC1.2

The adequacy of thyroxine replacement in hypopituitary patients

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Background
Hypopituitary patients with untreated growth hormone deficiency (GHD) have increased fat mass, dyslipidaemia and insulin resistance. Inappropriately low thyroxine doses in patients with central hypothyroidism (CH) may also promote such clinical features.
Objective
To examine metabolic outcome of thyroxine replacement in hypopituitary patients before and after GH replacement.
Method
One thousand and six hundred and two patients with GHD within KIMS (Pfizer International Metabolic Database) were studied before and 2 years after GH treatment. Patients with CH ($n=1087$) were divided into quartiles (Q1-4) for their L-thyroxine dose per kilogram bodyweight. The CH patients were compared with patients without CH (TSHsuff $n=515$) and the effect of dose quartiles were evaluated on weight, BMI, waist circumference (WC), waist/hip ratio (W/H), glucose, HbA1c, blood pressure, blood lipids, IGF-I and AGHDA score. Analyses were standardized for gender, age, peak GH level, age of onset and etiology.
Results
Women received higher L-thyroxine dose/kg per day than men, $P<0.001$. At baseline, weight, BMI, WC, W/H, HDL- and total-cholesterol were higher and glucose, IGF-I and AGHDA score lower in the CH group compared to TSHsuff group. CHQ1 (doses ≤ 1.08 μg/kg per day) and CHQ2 (doses <1.09–1.36 μg/kg per day) had increased weight, blood pressure and serum IGF-I and decreased AGHDA score compared with CHQ4 (doses ≥ 1.71 μg/kg per day), $P<0.05$. In addition, CHQ2 patients had increased WC and W/H compared to CHQ3 and CHQ4. Similarly, W/H was larger in CHQ1 compared to CHQ3. After 2 years of GH treatment CHQ1 lost weight, whereas the other quartiles gained, $P<0.05$. IGF-I increased more in the CH patients than in TSHsuff group but this was not affected by thyroxine dose/kg.
Conclusion
Subjects with doses of thyroxine ≥ 1.37 μg/kg per day resembled TSHsuff patients in metabolic endpoints more than CH patients with lower doses. The metabolic profile of CH patients with thyroxine doses ≤ 1.36 μg/kg per day suggests that they have signs of hypothyroidism and are inadequately treated.

OC1.3 – ESE Young Investigator Award

Limited effects of growth hormone replacement in adults with growth hormone deficiency after treatment for acromegaly

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Growth hormone deficiency (GHD) can occur after treatment for acromegaly. It is unclear whether treatment with recombinant human growth hormone (rhGH) in these patients is beneficial. Patients were randomized to either 1 year of rhGH replacement ($n=10$) or placebo followed by rhGH replacement for 1 year (delayed rhGH treatment, $n=6$). Sixteen patients (8 men, mean age 56 years) with GHD after treatment for acromegaly were studied. Study parameters were assessed at baseline and after 1 year of placebo ($n=6$, delayed treatment) and of rhGH replacement ($n=16$). The study parameters were: cardiac function, body composition, bone mineral density (BMD), fasting concentrations of lipids, glucose, insulin, C-peptide, PINP and β crosslaps, and quality of life using 4 different questionnaires (HADS, MFI-20, NHP and QoL-AGDHA). During 1 year of placebo ($n=6$), insulin concentrations decreased. In addition, left ventricular systolic function (fractional shortening and ejection fraction) decreased. Bone mineral density at the left hip decreased. Replacement with rhGH induced an increase of IGF-1 levels from 14.5 ± 6.4 nmol/l to 20.5 ± 6.5 nmol/l ($n=16$, $P=0.001$). However, rhGH did not alter any of the parameters of cardiac function, lipid and glucose metabolism, body composition or QoL. PINP and β crosslaps levels increased ($P=0.005$ and $P=0.021$, resp.), paralleled by a small but significant decrease in BMD of the left and right hip during rhGH replacement. In this trial, no beneficial effects of rhGH replacement in adults with GHD after treatment for acromegaly on cardiac function, lipid and glucose metabolism, body composition, bone mineral density or QoL could be observed.

OC1.4

Pasireotide (SOM230) effectively reduces pituitary tumor volume in patients with active acromegaly: preliminary 6-month results from a phase II extension study

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Introduction
In a recent 16-week Phase II study in patients with *de novo*, persistent or recurrent acromegaly, pasireotide effectively controlled GH and/or IGF-I levels in 56% of patients, and reduced tumor volume by >20% in 39% of patients. We present preliminary 6-month results from the ongoing extension phase of this study.
Methods
This extension study enrolled patients who achieved biochemical control (GH ≤ 2.5 μg/l and normalized IGF-I), or clinically relevant improvement, during 12 weeks' pasireotide treatment in the core study. Patients received pasireotide at the dose (200, 400 or 600 μg sc bid) at which biochemical control

was achieved in the core study, with dose adjustments up to 900 μg sc bid if required. Patients continued treatment for as long as benefit was derived. Pituitary MRI scans, acquired at months 0, 3 and 6, included T1-weighted sagittal and coronal, enhanced and unenhanced sequences, digitally assessed at a central laboratory. Tumor volume was calculated from coronal enhanced scans, as the sum of the tumor area on each image, multiplied by the effective section thickness.
Results
Thirty patients entered the extension study. Of 26 patients who received pasireotide for at least 6 months, 65% achieved GH $\leq$2.5 μg/l and/or normalized IGF-I, 62% had normalized IGF-I and 42% achieved GH $\leq$2.5 μg/l. Nineteen patients had both baseline and per-protocol follow-up MRI scans at 6 months. Mean tumor volume reduction at 6 months was 18.1$\pm$4.6% S.E.M. for all 19 patients. In 10 of the 19 patients (53%), tumor volumes decreased by a mean of 33.4$\pm$4.2% (20% measurement error threshold).
Conclusions
These preliminary results show that pasireotide significantly reduced pituitary tumor volume in 53% of patients after 6 months of treatment, and reduced GH and/or IGF-I levels in 65% of patients, including those resistant to prior surgical or medical therapy.

OC1.5

Cardiac performance after long-term treatment with Pegvisomant in patients with acromegaly: a radionuclide angiography study

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GH and IGF-I excess causes a specific cardiomyopathy, often complicated by diastolic and systolic dysfunction until heart failure. The aim of this study is to evaluate the effects of long-term treatment with pegvisomant on cardiac performance in acromegalic patients. Twelve patients (4 men and 8 women, age 29–58 years) entered the study. A radionuclide angiography at rest and during exercise was performed at baseline and after 18 months of treatment with pegvisomant with evaluation of heart rate (HR), systolic (SBP) and diastolic (DBP) blood pressure, peak filling rate (PFR), peak ejection rate (PER), PFR/PER ratio and ejection fraction (EF), both at rest (R) and during exercise (E). All patient received an initial dose of 10 mg/day of pegvisomant, then increased of 5 mg/day every 6 weeks on the basis of IGF-I levels until their normalization, or achievement of the maximal dose of 40 mg/day. At baseline, R-EF was normal in all patients whereas percent increase of EF after exercise was normal in 10 of the 12 (83.3%) patients. At the last follow-up, E-HR (130.7$\pm$17.4 vs 107.5$\pm$18.5 vs bpm, $P=0.02$), E-DBP (100.0$\pm$12.6 vs 86.2$\pm$4.3 mmHg, $P=0.01$) and E-PFR (3.98$\pm$1.42 vs 2.66$\pm$0.7 SV/s, $P=0.01$) were significantly reduced compared to baseline. No significant difference was found in E-SBP and E-PER. Moreover, although no significant difference was found in EF both at rest and after exercise, a significant improvement and normalization of the EF response to exercise was found in the two cases in which it was impaired at the study entry. Conversely, one patient showed a significant worsening of EF response to exercise. In conclusion, long-term treatment with pegvisomant improves cardiac performance, mainly ameliorating the response of heart rate and blood pressure to exercise.

OC1.6

The metabolic effects of ghrelin and glucocorticoids are mediated by AMP-activated protein kinase (AMPK) and endogenous cannabinoids

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Ghrelin, cannabinoids and glucocorticoids have all orexigenic and widespread metabolic effects. AMPK is a major controller of many metabolic processes. We have studied the effects of ghrelin and glucocorticoids and their interaction with endocannabinoids using cannabinoid-receptor-1 (CB1) knock-out mice and CB1 antagonist-treated mice, and using tissue samples from patients with Cushing's syndrome and from a rodent model of Cushing's syndrome. AMPK activity and downstream targets and endocannabinoid content were studied. Electrophysiological changes in response to ghrelin and CB1-antagonists were recorded using hypothalamic cells. Hypothalamic AMPK activity was significantly increased by ghrelin and this effect was lost in CB1-KO or with CB1-antagonism. We also observed an increase in hypothalamic endocannabinoid content in wild-type mice, effect that was blocked by genetic or pharmacologic inhibition of CB1. Electrophysiology studies confirmed the role of CB1 in mediating the effects of ghrelin. In adipose and liver tissue ghrelin inhibited AMPK, and again this effect was blocked by CB1 antagonism. Corticosterone-treated rats showed many features of the metabolic syndrome including significantly higher insulin, leptin, lipid levels as well as an increase in visceral fat weight. AMPK activity was higher in the hypothalamus and significantly lower in visceral fat and heart. In addition, we showed an increased hypothalamic cannabinoid content with glucocorticoids, suggesting that the well-known orexigenic effect of cortisol is mediated by cannabinoids and AMPK activation. Human fat tissue samples from Cushing's patients have reduced AMPK activity, explaining the lipogenic effect of cortisol in the human. These data were confirmed in *in vitro* cultured human adipocytes where 24 h dexamethasone treatment inhibited AMPK activity and upregulated mRNA expression of lipogenic genes. It is proposed that the metabolic hormones ghrelin and glucocorticoids markedly influence AMPK activity and this effect plays an important role in their orexigenic, diabetogenic and lipogenic actions: endocannabinoids appear to mediate many of these effects.

OC1.7

Intermittent high glucose concentrations reduce neuronal precursor proliferation by altering the IGF system: the involvement of the neuroprotective factor seladin-1

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The exposure of cells to high glucose concentrations is considered a determinant of diabetic neuropathy. Conversely, members of the Insulin-like Growth Factor (IGF) system are well known neurotrophic factors. Here, we investigated the effects of constant and intermittent high glucose concentrations on IGF-I and IGF Binding Proteins (IGFBPs) in human neuroblast long-term cell cultures (FNC). We first demonstrated that FNC express the IGF-I receptor, and express and release in the culture medium IGF-I, IGFBP-2 and IGFBP-4. IGF-I secretion was significantly increased by the exposure to 17β-estradiol (10 nM), in agreement with the reported cross-talk between the IGF system and estrogen in neuronal cells. FNC treatment with IGF-I (100 nM) increased the release of IGFBP-2, that is known to facilitate the binding of IGF-I to its receptor, whereas it reduced the release of IGFBP-4, that is considered an inhibitor of the biological actions of IGF-I. In addition, IGF-I stimulated FNC cell proliferation in a dose-dependent manner (1–100 nM). Previous data showed that 17β-estradiol increases the expression of the Alzheimer' disease related gene *seladin-1*, which acts as a pro-survival factor for neuronal cells. Similarly, here we observed that also IGF-I (10 nM) significantly increased the expression of this gene. In contrast with the effects of IGF-I, the exposure to intermittent (20/10 mM), but not stable (20 mM), high glucose concentrations inhibited FNC growth, and determined a decreased release of IGF-I and IGF-BP2. In addition, high glucose decreased the expression of seladin-1. In conclusion, our results suggest for the first time that intermittent high glucose concentrations, similar to those observed in poorly controlled diabetic patients, may contribute to the development of diabetic neuropathy by interfering with the trophic effects exerted by the IGF system, which might be mediated, at least partially, by the pro-survival factor seladin-1.

OC1.8

Differential sensitivity of men and women to anorexigenic and memory improving effects of intranasal insulin

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Background
Central nervous insulin is critically involved in the regulation of body weight and memory processing. Long-term administration of intranasal insulin reduces body weight in men but not in women while improving hippocampus-dependent memory processing in both genders. Here, acute effects of intranasal insulin on food intake and memory functions were studied in men and women.
Methods
Thirty-two healthy, normal-weight subjects (14 men, 18 women) were intranasally administered 160 IU regular human insulin or vehicle before performing a hippocampus-dependent 2-D-object location task, a working memory task (digit span) and a hippocampus-independent mirror tracing task. Subsequently, food intake from an ad libitum breakfast buffet was measured.
Findings
Insulin treatment decreased food intake in men but not in women (difference to placebo condition, men: -192.57 ± 78.48 kcal, $P<0.03$; women: 18.54 ± 42.89 kcal, $P>0.67$). In contrast, hippocampus-dependent memory and working memory were improved in women ($P<0.03$, $P<0.05$, respectively) whereas men did not benefit from acute insulin treatment ($P>0.17$, $P>0.20$). Performance on the hippocampus-independent mirror tracing task was not affected by insulin in both sexes.
Interpretation
In accordance with animal data, our results indicate that men are more sensitive than women to the acute anorexigenic effect of central nervous insulin signaling whereas insulin's beneficial effect on hippocampus-dependent memory functions are more pronounced in women. Our findings provide first support for the notion of a fundamental gender difference in central nervous insulin signaling that pertains to the regulation of energy homeostasis and memory functions.

OC1.9

Oxytocin alleviates the neuroendocrine and cytokine response to bacterial endotoxin in healthy men

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Oxytocin is a hormone and neurotransmitter with antiinflammatory and neuroimmune-modulatory properties. Physiological states of elevated plasma oxytocin levels are associated with reduced hypothalamic–pituitaty–adrenal response to different stressors. We examined the effect of exogenous oxytocin administration on bacterial endotoxin induced innate immune responses in humans. Ten healthy men received in a randomized, placebo-controlled, cross-over design (1) placebo, (2) oxytocin (1 pmol/kg per min i.v. for 90 min), (3) LPS (2 ng/kg i.v.) and (4) LPS + oxytocin. Oxytocin administration resulted in a significant transient suppression of the LPS-induced release of ACTH, cortisol, TNF-alpha, IL-1ra, MIP-1alpha, MIP-1 beta and IP-10 after endotoxin challenge in healthy volunteers. The increases of plasma procalcitonin (diagnostic and prognostic marker in sepsis), IL-4, IL-6, MCP-1 and VEGF were abolished throughout the study. *In vitro*, oxytocin (at concentrations of 10 pM–100 nM) had no impact on the effect of LPS in releasing TNF-alpha, IL-6 and MCP-1 in monocytes and primary blood mononuclear cells from healthy human donors. In summary, oxytocin decreases the neuroendocrine and cytokine activation caused by bacterial endotoxin in men. We exclude a direct action of oxytocin in peripheral monocytes and T lymphocytes and discuss the possibility of a pharmacological modulation of the cholinergic antiinflammatory pathway. Further studies are needed to establish a potential role of oxytocin in the therapy of inflammatory diseases, sepsis and malignancies associated with high VEGF levels.

Thyroid–OC2

OC2.1

Analysis of early gene expression in mouse thyroid development

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Background
Defective thyroid gland development occurs in 80% of congenital hypothyroidism (incidence of 1:3000–1:4000). Only <5% have been shown to be caused by molecular genetic defects in few transcription factor genes (Nkx2.1, Nkx2.5, Foxe1, Pax8, Hhex) which are known to play a role in thyroid gland development. Therefore, other genes with a critical role in early thyroid development are likely to be involved in the pathogenesis of thyroid dysgenesis.
Methods
We screened a whole-mount *in situ*-hybridization database for positive signals in thyroid precursor cells. This database contains the amount of about 3000 different gene probes hybridized on whole mouse embryos aged 9.5 days (E9.5). Positive probes where re- hybridized on histological slices of E 9.5 as well as E10.5, E11.5, E12.5, and E15.5. As a second method, RNA was isolated of thyroid precursor cells of 9.5 days old mouse embryos collected by lasermicrodissection. RT-PCR and sequencing was performed.
Results
Out of 10 positive patterns, we could prove expression in thyroid precursors of 1 gene using slice hybridization. We got positive signals in thyroid precursors at stage E9.5, E12.5, E15.5. We got negative signals at stage E10.5, E11.5. This was validated by RT-PCR. A PCR product of cDNA of E9.5 thyroid precursors could be amplified and sequenced. A PCR product of surrounding tissue was also amplified, but showed a different size. There was one exon missing in the sequence of this PCR product.
Conclusion
We identified a new gene not known so far to be expressed in thyroid precursor cells. The pattern of appearance at stage E 9.5, disappearance at E10.5 and E11.5 and reappearence at E12.5 has not been described for another gene before. This may suggest a special role of the gene and its transcript in different stages of thyroid development. Furthermore the different sizes of the PCR-products (thyroid and surrounding tissue) points to the presents of thyroid precusor specific splice-variants.

OC2.2

Identification of 25 novel NKX2-1 gene mutations in 100 patients with broad spectrum of brain and thyroid dysfunctions

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Objective
The NKX2-1 gene, also known as TITF-1, TTF-1 or T/ebp, is a member of the homeodomain-containing NK-2 transcription factor gene family and expressed in early development of thyroid, lung and forebrain. Initial screening of patients with isolated congenital hypothyroidism failed to show mutations. The first human heterozygous deletion affecting the NKX2-1 gene to be reported was a neonate with respiratory failure, primary hypothyroidism and neurological signs matching the developmental defects seen in NKX2-1 −/− mice. To date, few NKX2-1 gene mutations were identified in patients matching this highly variable phenotype. Here, we report the systematic molecular screening analysis of the NKX2-1 gene locus of 100 patients with thyroid dysfunction combined with movement disorders and pulmonary affection aiming to delineate the clinical spectrum of NKX2-1 deficiency.
Methods
DNA of all patients was analyzed by PCR followed by direct sequencing. In addition, high-resolution region-specific array-CGH was applied. Furthermore, functional *in vitro* analysis of missense mutations was performed to identify loss-of-function.
Results
In our series, 25 novel alterations were identified, i.e. 7 heterozygous deletions and 18 intragenic pointmutations. Three missense and two splice site mutations were shown to alter gene function by abolished DNA-binding capacity. All remaining cases were nonsense mutations resulting in prematurely terminated or elongated protein sequences. Genotypic heterogeneity was seen, as neither a

common microdeletion nor a mutational hot spot could be revealed. Analyzing the clinical spectrum, NKX2-1 variations were most likely to occur in patients with choreoathetosis and hypothyroidism (38.5%) and less frequent in patients with neurological, thyroid and pulmonal dysfunction (28.3%) or separate choreoathetosis (12.5%).
Conclusion
NKX2-1 deficiency is highly variable in phenotype and genotype as seen in the newly identified 25 individuals. NKX2-1 gene analysis seems to be reasonable if applied in patients with two or three clinical findings, respectively, i.e. hypothyroidism, choreathetosis and pulmonary dysfunction, but also in isolated choreoathetosis.

OC2.3

Global proteomic analysis in human thyroid proliferative/neoplastic primary culture lines

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Proteomics provides an avenue to understand the interaction between the functional pathways within a cell and its environmental milieu, independent of any changes at the RNA level. There are only a few studies in thyroid diseases using proteomics; variability in specimen content in fibrous versus follicular tissue plus the presence of other types of cells (blood, lymphatic) makes difficult to apply global proteomic to surgery pieces; commercial cell-lines of thyroid cancer were not carefully maintained to preserve thyroid phenotype. We have developed a thyroid-tumour bank of thyrocytes in culture from surgery pieces obtained in our Hospital (CHUS) with more than 40 primary thyroid cultures (BANTTIC, Bravo, Oncogene 2003; Bravo, Clin Cancer Res 2005): from normal thyroid (NT), follicular adenomas, (T-FA), and papillary carcinomas (T-PC); they maintain expression of thyroid markers such as thyroglobulin or TSH-R.
In this study, we want to standardize a method to study proteins expressed differentially between normal samples or benign pathologies (NT, T-FA) and differentiated papillary carcinomas (T-PC2 and T-PC3) from the BANTTIC.
Soluble proteins of the cultures were suspended in Prot Buffer (7 M urea, 2 M thiourea, 5% CHAPS). Fifty micrograms of proteins were passively rehydrated into 11 cm pH gradient strips (IPG 4-7L) and focused for 9000 V. Second-dimension separation was performed using 10% acrilamide-bisacrilamide gels and detection with silver stain. Spots were picked and digested with trypsin. For matrix-assisted laser desorption/ionization-mass spectrometry (MALDI-ToF Bruker RIV) analysis, the peptide digest mixture was directly spotted on the target plate together with a-cyane.
We have indentified common proteins in all cultures to use as controls: some belong to the cytoskeleton (beta-actin, tubulin, vimentin), others are metabolic enzymes (ATP sintase, casein-a-s1). But the pattern of staining shows differences between the NT, and T-PC2 or T-PC3. We are performing an exhaustive analysis, in order to identify specific and consistent spots/proteins useful as markers of thyroid cancer.

OC2.4

Identification and enrichment of thyroid stem cells in anaplastic cell lines

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In the last years, a growing body of evidence supports the notion that tumors are organized in a hierarchy of heterogeneous cell populations with different biologic properties and that the capability to sustain tumor formation and growth exclusively resides in a small proportion of tumor cells, termed cancer stem cells (CSC). The existence of CSC was first proven in the context of acute myelogenous leukaemia, and subsequently verified in brain, breast, colon and prostate cancers. These studies have shown that CSC are responsible for tumor formation and progression and share with stem cells the key feature of self-renewal. Our aim was to identify and characterize CSC in different anaplastic thyroid cancer cell lines. For this purpose, we analysed the expression of stem cell markers, such as CD133, OCT4, c-KIT, THY-1, NANOG and GATA-4, in cultured ARO, KAT-4, KAT-18 and FRO cell lines, simultaneously with specific thyrocyte markers (TPO, thyroglobulin and CK-19), by flow cytometry and real time PCR. Only two cell lines, ARO and KAT-4, showed CSC positive for CD133 ($44 \pm 12\%$) and OCT4 ($30 \pm 15\%$) while the other CSC markers were absent in all the examined cell lines. Furthermore, $CD133^+$ stem like cells sorted by specific magnetic beads (Miltenyi Biotec) ($>90\%$), were analysed for their growing capabilities in basal conditions and without fetal bovine serum in the presence of specific stem cell growth factors (bFGF and EGF). Our data showed that $CD133^+$ stem like cells were characterized by elevated proliferation, self-renewal features and by expression of stemness genes when compared with the $CD133^-$ cells. In conclusion, we report the identification of CSC population in ARO and KAT-4 anaplastic cell lines characterized by their $CD133^+/OCT4^+$ phenotype. The identification of tumorigenic thyroid CSC could provide new insights into the anaplastic thyroid tumorigenic process and possibly bear great therapeutic implications.

OC2.5

Fetal cell microchimerism in papillary thyroid cancer: a possible role in tumor damage and tissue repair

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Fetal cells enter the maternal circulation during pregnancy and can persist in the maternal blood or tissues for decades, creating a physiological microchimerism. Since papillary thyroid cancer (PTC) is more frequent in female gender and it is the second more frequent tumor during pregnancy, the role of persisting microchimeric cells has been investigated. Tumour tissue specimens were obtained from 62 women with PTC, 41 of whom had at least one male child before the diagnosis of PTC. Male cells were identified by PCR ELISA studies on a male specific gene, the sex-determining region Y (SRY) and were further characterized by FISH. Male DNA was detected in the pathological tissue of the 17% of women who had a male pregnancy before the diagnosis. By FISH analyses, the total number of microchimeric male cells was significantly higher in neoplastic tissues than in normal sections. By immunoFISH, a technique combining FISH and immunohystochemistry in the same section, male cells expressing thyroglobulin were found in tumor and normal tissues, while male microchimeric cells stained with the CD45 common leukocyte antigen were detected only in pathological sections. Microchimeric cells negative for both markers were detected in tumour and in normal tissues, but they were significantly more frequent in tumoral samples.
In conclusion, the present study shows for the first time the presence of fetal cell microchimerism in women affected with PTC. $CD45^+$ male cells found in the neoplastic tissues might be committed to destroy the tumoral cells, while Tg^+ cells could have a repair function. Finally, microchimeric cells negative either for CD45 and for Tg could have staminal properties able to transdifferentiate in different cellular types. The whole of the results obtained suggests a protective role of microchimerism in thyroid cancer, though a possible role in carcinogenesis of persistent microchimeric cells cannot be excluded.

OC2.6

Ga-68-Dotatoc PET detects somatostatin receptors in normal thyroids and various thyroid pathologies

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Recent reports confirmed increased SSTR expression in the thyroid in some cases of Graves' disease and hot nodules. It is still not known, whether SSTRs can

frequently be found in other thyroid pathologies or normal thyroid glands. Therefore, we performed a systematic analysis of Ga-68-Dotatoc PET Scans for their thyroid image.
Eighty-three consecutive patients undergoing Ga-68-Dotatoc PET were analysed for their relative thyroid uptake by ROI technique for each thyroid lobe separately. Using a 1 cm thick coronal slice including highest thyroidal uptake, target-background-ratios (TBRs) were calculated. TSH, fT3, fT4 and anti-TPO were measured, the thyroid specific history was recorded and thyroid ultrasound was performed. 99mTc scintigraphy was used in cases of a TSH <0.4 mU/l or sonographically detected nodes.
Thyroid lobes without pathology ($n=62$) show a significantly higher uptake as compared to the same region after lobe excision ($n=24$) ($P<0.001$). Compared to normal thyroid lobes, goitrous lobes ($n=22$) in mean show a slightly increased uptake ($P=0.025$). Markedly increased uptake was found in thyroid lobes with hot nodules ($n=2$, TBR $=9.7$ and 6.4) and in disseminated autonomy.
PET scans showed a high uptake in 3 of 5 patients with active Hashimoto's disease.
Results confirm a high SSTR density in hot nodules and disseminated autonomy. However, normal as well as thyroids with non-toxic nodules and goiters show a clearly detectable SSTR expression with large variability. Intense tracer uptake was found in only few cases of structurally and functionally normal thyroid lobes. Like Graves' disease, some cases of active Hashimoto's disease show a high SSTR density.

OC2.7

Impact of resistance to thyroid hormone on the cardiovascular system in adult humans

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Background
Resistance to thyroid hormone (RTH) is an inherited condition of reduced tissue responsiveness to thyroid hormone (TH), biochemically characterized by elevated serum TH concentrations, with inappropriate, non-suppressed thyrotropin levels (TSH). It is caused by point mutations in the ligand binding domain of the TH receptor (TR) β gene, and the mutant TR interferes with the function of the normal TRs (dominant negative effect). The clinical manifestations of RTH are highly variable and the impact of RTH on the cardiovascular system has been poorly investigated.
Aim
We compare the cardiovascular characteristics of 16 patients with RTH with those of euthyroid healthy controls to define the cardiovascular involvement in RTH syndrome.
Patients and methods
Sixteen RTH patients (8 males; age: 32 ± 12 years, range: 19–63) and 16 controls (9 males; age: 33 ± 5 years, range: 24–42) were included in the analysis. For each patient, clinical data (age, gender, body mass index, blood pressure, heart rate, cardiovascular symptoms) and assessment of thyroid status were recorded. An echocardiographic evaluation was performed by a single investigator, blinded to the subject's clinical data.
Results
RTH patients did not show cardiovascular symptoms (palpitations, dyspnea at rest and after exercise). Heart rate was comparable to the control group whereas arterial pressure was higher than controls. RTH patients showed mean interventricular septum diastolic thickness (IST) and mean left ventricular posterior wall diastolic thickness (LVPWT), significantly lower than the controls with consequent significant decrease of the mean LV mass and LV mass indexed by body surface area. RTH patients also showed abnormalities of myocardial relaxation as indicated by the significant increase of peak-A and consequent reduction of E/A ratio. Finally, in RTH patients, systemic vascular resistance was significantly increased compared to those of the control group.
Conclusions
Our results suggest an altered and inefficient cardiovascular action of TH in RTH patients.

OC2.8

Graves' ophthalmopathy in patients treated with radioiodine I-131

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Introduction
Radioiodine treatment of hyperthyroidism of ophthalmopathy (GO) patients may cause or aggravate GO (in some 15%). We evaluated the activity and severity of ophthalmopathy in patients who acquired GO following radioiodine therapy.
Materials and methods
Over the years 2003–2005, 1500 hyperthyroid patients were treated with radioiodine at our Clinic. Of these, 50.9% suffered from Graves' disease. Following their radioiodine treatment, in 30 females and 7 males (i.e. 5% of Graves' patients) of mean age 53.9 ± 11.6 years, onset of GO was observed within 1-year post-treatment follow-up. I-131 treatment was only offered to patients with NOSPECS score <3 and CAS <3. Prior to their treatment, mean TSH concentration was 0.24 ± 0.58 μU/m, mean 24-h I-131 uptake $54\pm14.6\%$, and mean I-131 activity 496 ± 141 MBq.
Results
Six months post I-131 treatment, 68% of patients were cured while 32% required further methimazole medication. Over the 1-year follow-up all these patients were maintained euthyroid. In patients who developed GO after I-131 treatment, mean values of hTRAb and NOSPECS score were 24.3 ± 18.8 U/l and 4.9 ± 1.5 points, respectively, at the time of GO onset. Patients were qualified for SoluMedrol pulse therapy (8.0 g) and subsequent radiotherapy (20 Gy). Patients reported for control 1, 6 and 12 months post therapy. Mean concentration of hTRAb and NOSPECS score at 1, 6 and 12 months were: 11.8 ± 10.1 U/l and 4.0 ± 1.6; 14.5 ± 15.6 U/l, 3.4 ± 1.3; and 7.7 ± 7.6 U/l and 2.5 ± 1.2, respectively. Positive correlation between hTRAb and NOSPECS score was observed over the control period. IL-6 and IL-2 concentration prior to treatment and 1 month post treatment remained elevated and did not correlate with hTRAb nor with NOSPECS score.
Conclusions
Five percent of Graves' disease patients developed severe GO following radioiodine treatment, thus association between radioiodine therapy and severe ophthalmopathy cannot be excluded. Preventive administration of glucocorticoids should be recommended in patients with Graves' disease, even with mild ophthalmopathy.

OC2.9

Subclinical hypothyroidism in older patients: an analysis of natural course and risk factors for the thyroid failure

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We aimed to evaluate the risk factors for the development of definitive thyroid failure, to analyze the natural course of subclinical hypothyroidism and to quantify the incidence rate of overt hypothyroidism in elderly patients.
Two hundred and fourteen patients (186 women and 28 men) over age 60 years with subclinical hypothyroidism and no previous history of thyroid disease were prospectively studied. Subjects were followed up for 6–72 months (mean, 31.7 months) with repeated determinations of TSH and free T_4. Fifty-eight patients (27.10%) developed overt hypothyroidism, and 81 (37.85%) showed normalization of their TSH values. The incidence rate of overt hypothyroidism was 9.91 cases per 100 patient-years in the whole population, and 1.76, 19.67, and 73.47 cases per 100 patient-years in subjects with initial TSH values between 6.0–8.9, 9.0–13.9, and 14.0–18.9 mU/l, respectively.
Kaplan–Meier analysis showed that the development of definitive thyroid hypofunction was significantly related to the presence of symptoms of hypothyroidism, goitre, positive thyroid antibodies ($P<0.05$), and mainly low normal free T_4 ($P<0.01$) and high TSH ($P<0.001$) concentrations at baseline. A stepwise multivariate Cox regression analysis showed that the only significant factor for progression to overt hypothyroidism was serum TSH concentration ($P<0.001$).
In conclusion, TSH concentration is the most powerful predictor for the outcome of spontaneous subclinical hypothyroidism in older. Subjects with mildly elevated TSH have a low incidence rate of overt hypothyroidism. We recommend follow-up with clinical and biochemical monitoring in these patients.

Diabetes and obesity – OC3

OC3.1

Interaction of hypothalamic G-protein-coupled receptors (GPCRs) involved in weight regulation

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Food intake is centrally regulated in hypothalamic nuclei. Peripheral hormonal signals activate their corresponding receptors in the *arcuate nucleus* (arc) and modulate the expression of pro-opiomelanocortin (POMC) and neuropetide Y (NPY)/agouti-related-protein (AgRP). Cleavage products of POMC stimulate melanocortin-4-receptors (MC4R) in the *paraventricular nucleus* (PVN) to inhibit food intake or stimulate MC3Rs in the arc to activate a feedback loop. Further more other neuropeptides or neurotransmitters are involved in hypothalamic regulation of body weight, which also act through GPCR coexpressed with melanocortin receptors in hypothalamic nuclei. The concept of homodimerization of GPCRs today is well Recently we could show homodimerization of MC4R.
In a systematic approach, we investigated the interaction of GPCRs that are expressed on the same neurons. We report the interaction of the MC3R and GHSR that are coexpressed on arcuate NPY/AgRP neurons. Mutations in both receptors were shown to influence body weight. We used two different methods to investigate GPCR oligodimerization: a sandwich-ELISA approach with differentially N- and C-terminally tagged receptors and the FRET acceptor photobleaching technique which allows monitoring of GPCR interaction in living cells. Both methods displayed a strong signal of MC3R/GHSR oligomerization. We could demonstrate a co-localization of the heterologously expressed receptors on the cell surface of living cells by confocal laser scanning microscopy. Functional analysis revealed that co-transfection of GHSR and MC3R increases the maximal secondary messenger answer after melanocortin stimulation in COS-7 cells compared to MC3R transfected alone. Additionally co-internalization of the dimer could be shown.
In conclusion, we demonstrate that GPCR from different subfamilies that are expressed on the same neuron and are involved in weight regulation are able to form receptor oligomers. These findings may provide a mechanistic basis of a functional interaction between melanocortin and ghrelin receptors and thereby widen our understanding of hypothalamic signaling pathways involved in weight regulation.

OC3.2

Adiponectin inhibits AMP kinase activity and increases insulin secretion in MIN6 cells

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Adiposity predisposes to insulin resistance and type II diabetes. Adipose tissue is an active endocrine organ, secreting adipokines with important physiological actions. Adiponectin is a recently discovered adipokine whose levels are, paradoxically, decreased in obesity despite the increase in adipocyte mass. Adiponectin suppresses triglyceride accumulation, increases fatty acid oxidation and activates AMP kinase (AMPK) in skeletal muscle, improving insulin signalling, and it suppresses glucose production and activates AMPK in liver. Hence, adiponectin is an insulin sensitizer in skeletal muscle and liver. Our aim here was to investigate the effects of adiponectin on pancreatic β-cell function and, in particular, on AMPK since, in these cells, AMPK activation causes inhibition of insulin secretion. We used MIN6 cells, a murine pancreatic β-cell line. Adiponectin (2 μg/ml) suppressed AMPK activity and caused an increase in insulin secretion. Insulin secretion requires an increase in free cytoplasmic Ca^{2+} ($[Ca^{2+}]_i$), and we examined the effect of acute (30 min) application of adiponectin on $[Ca^{2+}]_i$. Adiponectin caused a prompt increase in $[Ca^{2+}]_i$. The increases in $[Ca^{2+}]_i$ and insulin production were prevented by nifedipine, a blocker of L-type Ca^{2+} channels, and did not occur in Ca^{2+} free solution. The suppression of AMPK activity and increases in insulin and $[Ca^{2+}]_i$ induced by adiponectin were comparable to those evoked by high glucose stimulation (Table).

Table Responses expressed as % of AMPK activity, insulin and $[Ca^{2+}]_i$ in 3 mM glucose.

	pAMPK/tAPMK	Insulin	$[Ca^{2+}]_i$
Adiponectin (in 3 mM glucose)	48±10% (P=0.01)	303±51% (P=0.001)	142±14% (P=0.03)
25 mM Glucose	68±6% (P=0.01)	173±12% (P=0.001)	161±11% (P=0.01)

In conclusion, adiponectin has a profound direct effect on pancreatic β-cell function. Adiponectin suppresses the activity of AMPK, and increases insulin secretion by increasing $[Ca^{2+}]_i$ via influx across the plasma membrane. These results demonstrate that adiponectin can contribute to insulin secretion and reveal its potential as a novel therapeutic target against obesity-linked type II diabetes.

OC3.3

Regulation of lipolysis by corticotropin releasing hormone in brown adipocytes

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Corticotropin releasing hormone (CRH) and its related peptides, urocortins (UCN), are now emerging as critical regulators of energy balance and homeostasis. These peptides exert their effects through activation of two types of CRH receptors (CRH-R1 and CRH-R2). The role of these peptides as regulators of mammalian thermogenesis and their effects on brown adipose tissue (BAT) have been primarily attributed to modulation of brain centres controlling sympathetic outflow. However, direct effects of CRH and UCNs on brown adipocytes have not been investigated. In this study we have demonstrated that mRNA of both types of CRH-Rs is expressed in mouse BAT and in a brown adipocyte cell line (T37i). Immunofluorescence confocal microscopy demonstrated that CRH-Rs are not uniformly distributed over the cell membrane, but are also present in cytoplasmic compartments and around the nucleus, potentially indicating newly synthesised unprocessed receptor, internalised mature CRH-R or 'cytoplasmic' forms of specific CRH-R variants. Western blot analysis identified multiple immunoreactive proteins indicative of receptor heterogeneity and potentially differential post-translational processing. Exposure of T37i adipocytes to CRH led to significant increase in cAMP levels and glycerol release in a dose-dependent manner, via a PKA-dependent mechanism. Maximum CRH effects were observed at 1 nM, whereas CRH concentrations >10 nM has smaller or insignificant effect on cAMP and glycerol release. Further studies showed that CRH induces phosphorylation of hormone sensitive lipase (HSL) at Ser-563 and its subsequent translocation toward lipid droplets. This was associated with redistribution of perillipin on the lipid droplets. In conclusion, this is the first report to show a direct effect of CRH on brown adipocyte lipolysis. This signalling pathway appears to be inactive at high CRH concentrations, raising the possibility that excess CRH availability might modulate distinct biological effects of BAT.

OC3.4

Fatty acid-induced induction of TLR-4/NFκB-pathway in adipocytes links nutritional signaling with innate immunity

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Objectives
To study the direct effects of fatty acids (FA) and the involvement of the TLR-4/NFκB pathway with respect to the secretion of adipokines and chemokines from adipocytes.
Research design and methods
3T3-L1 adipocytes were stimulated with increasing doses of FA. The secretion of adiponectin, resistin and MCP-1 was measured by ELISA. NFκB p65 nuclear translocation and TLR-4 expression were investigated by Western blot analysis. NFκB-mediated effects were tested by a specific NFκB-inhibitor. TLR-4-induced

effects were analyzed by using a neutralizing TLR-4 antibody. The mRNA expression of adipokines in abdominal adipose tissue of rats fed a standard chow or a high fat diet was investigated by quantitative real time RT-PCR.
Results
FA are capable of stimulating adiponectin and resistin secretion. Concerning MCP-1 secretion, FA exert class-specific and differential effects. TLR-4 is induced during adipocyte differentiation and its expression is enhanced following fatty acid stimulation. The stimulatory effects of stearic and palmitic acid on MCP-1 secretion and of palmitoleic acid on resistin secretion are mediated via NFκB as demonstrated by using a soluble NFκB inhibitor. The stimulatory effects of stearic, palmitic and palmitoleic acid on resistin secretion and the stimulatory effect of stearic acid on MCP-1 secretion are mediated via TLR-4 as demonstrated by using a neutralizing TLR4 antibody. Adipose tissue mRNA expression and serum levels of adipokines did not differ in rats fed a high fat diet.
Conclusions
TLR-4 is induced during adipocyte differentiation and in response to stimulation with FA. FA can activate TLR-4 signaling and subsequent NFκB-activation in order to regulate adipokine and chemokine secretion from adipocytes. The present data provide a new molecular mechanism by which FA can link nutrition with innate immunity in a paracrine or autocrine manner.

OC3.5

Hormonal regulation of 11-β-HSD1 expression and activity in differentiated 3T3- L1 adipocytes

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Introduction
In humans, testosterone treatment affects body fat distribution and metabolism. The mechanisms involved in these processes are not yet fully understood. We investigated whether sex hormones modulate the expression and activity of type1 11-β-hydroxy-steroid-dehydrogenase (11β-HSD1). 11β-HSD1 catalyses the conversion of inactive cortisone into active cortisol, a key step for the development of functional adipocytes. Based on previous studies an inhibition of lipogenic activity of HSD1 would have been predicted.
Methods
Differentiating and mature 3T3-L1 cells were investigated for 11β-HSD1 expression and activity under the various doses of DHT, E2, insulin and liver X receptor (LXR) agonist. Intracellular fat accumulation was also assessed. Gene expression was analyzed by RT-PCR. 11β-HSD1 activity was estimated by interconversion of radiolabelled [3H]-cortisone into [3H]-cortisol after extraction, TLC separation and β-scanning. Lipid accumulation was evaluated using a modified procedure for serum triglycerides and Oil Red O staining.
Results
Form day 4 of differentiation, 3T3-L1 show a steady increase in 11β-HSD1 expression and activity that parallel fat accumulation. Treatment with DHT produced no reduction, but rather a weak increase, of 11β-HSD1 expression ($17.3 \pm 8.2\%$ DHT 10 nM versus control). Insulin positive effects on 11β-HSD1 activity were not antagonized by androgens. Conversely, E_2 showed a slight inhibition of the enzymatic activity with no significant changes in mRNA expression ($-9.3 \pm 13.9\%$, E_2 1 nM). LXR agonist strongly inhibited 11β-HSD1 expression (-64.3%) and activity (-45.1%) (LXR-A, 1 μM vs C).
Conclusion
In contrast to LXR agonists, androgens do not inhibit the availability of intracellular active glucocorticoids in mature adipocytes. The proposed explanation is that androgens sustain maintenance of a functional phenotype in mature adipocytes through the pro-differentiating effect of 11β-HSD1.

OC3.6

Transcriptional control of hepatic and systemic lipid metabolism by the nuclear receptor co-factor RIP140

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Given the importance of transcriptional regulation in metabolic control, we investigated the role of nuclear receptor co-factors in the liver, a key organ in energy homeostasis. Here we show in mice that the expression of receptor interacting protein 140 (RIP140) in the liver is induced in catabolic states like fasting and cancer cachexia. In contrast, chronic high fat diet feeding reduced RIP140 mRNA levels in the liver. Increased hepatic RIP140 in catabolic conditions was associated with an elevated hepatic triglyceride (TG) content (fatty liver) and decreased circulating TG levels, indicating a role of RIP140 in the transcriptional regulation of hepatic lipid metabolism. Indeed, an acute, liver-specific depletion of RIP140 through adenoviral-delivered shRNA expression decreased hepatic TG content and increased blood TG levels. The reduced expression of the lipid transporters caveolin and CD36 upon RIP140 knockdown might contribute to the observed decrease in liver TG content by reducing lipid influx into the liver. Interestingly, expression of SREBP-1c and FAS, genes involved in *de novo* lipogenesis were induced in RIP140-deficient livers, and consistently also hepatic VLDL-TG secretion was elevated. In addition, we observed a reduced expression of apolipoprotein A5 (ApoA5). ApoA5 has been shown to be involved in the control blood TG levels, at least in part, by accelerating the hydrolysis of triglycerides by lipoprotein lipase (LPL) for fatty acid uptake into adipose and muscle tissue. In agreement, we observed decreased LPL activity levels in white adipose tissue from mice with liver-specific RIP140 knockdown. Both, increased hepatic VLDL-TG secretion as well reduced LPL-dependent TG uptake by peripheral tissues might contribute to the observed elevation of serum TG levels. Our results suggest a crucial role of hepatic RIP140 in the metabolic control of processes associated with dyslipidimia in anorexia, cachexia and the metabolic syndrome.

OC3.7

Impact of metformin on gene expression, glucose uptake and lipolysis in adipocytes

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The biguanides metformin and phenformin enhance insulin sensitivity and improve glycaemic control in type 2 diabetes. Metformin is the most prescribed oral agent for type 2 diabetes but phenformin has been withdrawn because of its propensity to cause lactic acidosis. Metformin has a major effect in the liver where it reduces glucose output via AMPK activation. As the adipocyte is now considered to be a critical participant in whole-organism metabolic homeostasis we investigated metformin action in the murine 3T3-L1 adipocyte model. Treatment of cells with metformin for 2 h had no effect on subsequent basal or insulin-stimulated glucose uptake or on basal or isoproterenol-stimulated lipolysis, although phenformin significantly reduced insulin-stimulated glucose uptake and isoproterenol-induced lipolysis. AMPK activity was more strongly activated by phenformin than metformin. Moreover, phenformin markedly inhibited differentiation of preadipocytes, while metformin had no significant effect. To determine whether metformin might affect adipocyte functions not captured by targeted biochemical assays we undertook a global transcriptomic analysis of metformin-treated adipocytes. While 2 h metformin treatment did not induce any significant changes in mRNA expression, 12 h incubation significantly altered the abundance of 44 transcripts, including mRNAs encoding key enzymes involved in metabolism and protection from oxidative stress, and several transcription factors and transcriptional co-regulators. We conclude that, acute treatment with metformin has little impact on adipocyte function, but prolonged treatment results in significant changes in gene expression, including induction of genes implicated in response to cellular stress, suggesting that some of metformin's beneficial effects may be mediated through the modification of the stress response. In contrast, the striking effects of phenformin in adipocytes may contribute to its adverse metabolic profile.

OC3.8

Plasma endothelin as a biochemical marker of endothelial dysfunction in endocrine diseases with increased cardiovascular risk

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Background
The aim of the present study was to represent our summarized results of plasma endothelin-1 (ET-1) level determined in some endocrine diseases like diabetes, hyper- and hypothyroidism, Cushing's disease, acromegaly and male hypogonadism in which the existence of endothelial dysfunction and increased cardiovascular risk is proved.
Patients and results
In patients with type 2 diabetes ($n=34$), plasma ET-1 (0.9 ± 0.17 pmol/l) correlated with the degree of vascular complications and reached the highest level in hemodialysis diabetics (3.4 ± 0.38 pmol/l). ET-1 was significantly elevated in patients with hyperthyroidism (0.78 ± 0.11 pmol/l, $n=18$) and decreased to normal range after thyrostatic treatment (0.5 ± 0.1 pmol/l). However, in hypothyroid subjects ($n=20$) ET-1 did not differ from controls (0.49 ± 0.12 vs 0.46 ± 0.2 pmol/l). ET-1 level in patients with Cushing's disease was 3-fold higher (1.6 ± 0.8 pmol/l, $n=13$) than in healthy subjects, and decreased significantly after disease remission (0.73 ± 0.53 pmol/l). Hypersomatotropism in acromegaly ($n=28$) leads to significant elevation of ET-1 (1.24 ± 0.2 pmol/l). After treatment and normalization of IGF-1, ET-1 showed the normal concentration (0.39 ± 0.1 pmol/l). The low testosterone level in male hypogonadism ($n=33$) caused higher plasma ET-1 (0.95 ± 0.53 pmol/l). Patients with hypergonadotrophic hypogonadism had significantly higher ET-1 in comparison to hypogonadotrophic hypogonadism (1.05 ± 0.57 pmol/l vs 0.89 ± 0.53 pmol/l). In all groups no correlation was observed between the concentrations of ET-1, blood pressure, lipid status and plasma homocystein.
Conclusions
Our results clearly demonstrate that hyperthyroidism, hypercortisolism, hypersomatotropism and hypogonadism lead to activation of endothelin system. Elevated plasma ET-1 levels probably play a role in the pathogenesis of accelerated and early atherosclerosis development in this disorders.

OC3.9

Effect of baseline sample characteristics, comparator drug, co-interventions and rosiglitazone doses on the risk of myocardial infarction: multivariable lineal regression analysis

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Nissen's meta-analysis concluded that the use of Rosiglitazone (RSG) was associated with a significant increase in the risk of myocardial infarction. However, none of the analyzed studies took into account the cardiovascular events as primary outcomes, neither were controlled by cardiovascular risk factors.
Objectives
1. To know if there is an association between RSG myocardial infarction OR and the magnitude of the difference in baseline serum lipids and HbA1c in intervention groups.
2. To know the effect of RSG doses, comparator drug and co-interventions in RSG's myocardial infarction OR.
Methods
We analyzed the same studies included in Nissen's meta-analysis. For each study we recorded myocardial infarction incidence, comparator drug, co-intervention, RSG doses, the difference in baseline serum lipids and HbA1c in intervention groups and the difference in serum lipids and HbA1c change attributed to intervention. We modeled the RSG's myocardial infarction OR with the variables detailed before by bivariate and multiple lineal regression with Stata/SE 10.0 software (Stata Corp LP, 4905 Lakeway Drive College Station Texas 77845, USA).
Results
Myocardial infarction OR increased if comparator was placebo, co-intervention was insulin and RSG doses was 2 mg. We didn't find an association between myocardial infarction OR and the difference in baseline serum lipids and HbA1c in intervention groups. When we studied the myocardial infarction OR and the difference in serum lipids and HbA1c change attributed to intervention by bivariate lineal regression, only an increase in serum triglicerides could predict an increment in myocardial infarction OR. When we modeled Rosiglitazone's myocardial infarction OR with the difference in baseline serum lipids and HbA1c in intervention groups r^2 was 0.49.
Conclusion
The effect of Rosiglitazone on the risk of myocardial infarction can change with its doses and co-interventions. The adverse change in serum lipids induced by RSG didn't affect the magnitude of myocardial infarction risk.

Bone and adrenal – OC4

OC4.1 – ESE Young Investigator Award

Inactivation of the Carney complex (CNC) gene 1 (PKA regulatory subunit 1A, *PRKAR1A*) by interference RNA alters multiple signaling pathways and decreases apoptosis

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The cAMP signaling pathway plays an important role in cell proliferation and differentiation, and can be altered at multiple levels in endocrine tumors. Its central component is the protein kinase A (PKA). Inactivating mutations of *PRKAR1A* are observed in CNC (a dominant autosomal hereditary disease responsible for primary pigmented nodular adrenocortical disease, cardiac myxoma and lentiginosis). Most *PRKAR1A* mutations lead to mRNA unstability and protein degradation. To study the consequences of *PRKAR1A* mutations we have developed an interference RNA approach in the human embryonic kidney (HEK293) and adrenocortical (H295R) cell lines that allow a 50–80% decrease of the R1A protein.
PRKAR1A inactivation in these two cell lines increases by 2-fold the forskolin (FK) stimulation of a PKA-dependent luciferase reporter construct (CRE-Luc). It also increases rapidly endogenous *NURR1* (an ubiquitous target of CREB) expression after 1h of FK stimulation (HEK293: x2.2; H295R: x1.2). As expected, the PKA enzymatic activity is stimulated after *PRKAR1A* inactivation (basal activity +12%, and after FK +20%). But interestingly, *PRKAR1*A inactivation exerts various effects on others signaling pathways more frequently involved in tumorigenesis. We have observed an activation of MAPK (proliferation) and PI3K/AKT (cell survival) pathways (x2 of P-ERK1/2 and x2 of P-AKT). Moreover, TGFbeta pathway (known to inhibit adrenal steroidogenesis and to induce apoptosis in numerous cell types) is altered by decreased expression of SMAD3 (−40%). This leads to a decreased response to TGFbeta stimulation (−24%) of a SMADs reporter construct (CAGA-Luc). SMAD3 expression is also inhibited by ACTH *in vivo* in the mice adrenal and by FK *in vitro* in H295R cells. Finally, *PRKAR1A* inactivation leads to resistance to apoptosis induction by TNFalpha (−14% of apoptosis cells with 20 ng/ml per 24 h and −26% of dead cells with 100 ng/ml per 24 h).
Tumorigenesis due to *PRKAR1A* mutations could results from effects on multiple signaling pathways and apoptosis dysregulation.

OC4.2 – ESE Young Investigator Award

Reduced fertility rates and high prevalence of testicular adrenal rest tumors (TART) in male patients with congenital adrenal hyperplasia

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Objective
To evaluate reduced fertility rates and their possible causes in a cohort of well controlled male adult patients with congenital adrenal hyperplasia.
Methods
We clinically assessed 22 male patients with congenital adrenal hyperplasia (15 salt wasting form, 7 simple virilising, age 18–49) according to their hormonal control. Further, we performed testicular ultrasound, MRI of the testis and a sperm count of each. As laboratory markers 17-OHP in serum and saliva were determined as well as adrostendione, testosterone, pregnatiol in 24 h urine, LH, FSH, ACTH, renin concentration and estradiol.
Results
All patients had a pathological sperm count with reduced fertility (1 patient with azoospermia, 7 patients with oligospermia, all with pathological sperm morphology and sperm motility). TART prevalence was 14/22 (8 SW, 6 SV), none of those were palpable. Poor therapy control with elevated 17-OHP in

serum/saliva and/or pregnantriol in urine could be shown in 4 patients, the other 18, however, were well controlled or even had suppressed adrenal androgen levels. Ten of the well controlled patients had TARTs. Sperm concentration correlated with testicular volume. About 10/22 patients also had reduced Leydig cell function according to low testosterone levels.
Conclusion
TARTs in CAH patients are an underestimated problem and are probably the most important risk factor for reduced fertility rates in male CAH patients. Regular testicular ultrasound from childhood on is required.

OC4.3 – ESE Young Investigator Award

BMP dependent modulation of adrenocortical growth and function

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Bone morphogenic proteins (BMPs) have been demonstrated to impact tumorigenesis in a variety of tumors. As for the adrenal cortex, BMP6 has been implicated as an important modulator of aldosterone secretion. To screen for alterations of BMP dependent pathways in adrenal tumorigenesis we performed gene profiling experiments. By comparing human adrenal carcinoma (ACC) against normal adrenal glands (Co) we detected a down-regulation of various BMPs (e.g. BMP2, BMP5) which was further validated by qPCR (Co vs ACC, BMP5, 100±29.7 vs 6.1±1.4%; BMP2, 100±17 vs 35.1±1.2%). Similar expression patterns were seen in two human ACC cell lines (NCIh295R, SW13), which were therefore used as an *in vitro* model for further studies of the impact of BMP on adrenal function. Integrity of the pathway could be demonstrated by incubation with recombinant hBMP2 and 5, which induced phosphorylation of SMAD1/5/8 and subsequent increase of ID protein expression levels in a dose dependent manner. Co-incubation with the physiological BMP antagonist Noggin neutralized these effects. On a functional level, incubation with BMP2 significantly diminished cell proliferation in a dose dependent manner (untreated versus BMP2; 10, 50, 100, 300 ng/ml, 100±8.9 vs 78.9±4.5 vs 66.2±2.3 vs 55.1±4.6%). Moreover, BMP2/5 treatment resulted in a decrease of forskolin stimulated cortisol production (untreated versus BMP2, 151.3±6.3 vs 48.3±1.2 nmol/l; untreated versus BMP5 438.0±31.2 vs 254.3±6.3 nmol/), which was accompanied by down-regulation of 3ßHSD (untreated versus BMP2, 100±14.3 vs 11.2±0.2 vs BMP5, 8.5±0.8) and MC2-R expression (untreated versus BMP2, 100±0.8 vs 17.3±0.5 vs BMP5, 10.8±0.8). Taken together, we demonstrate that loss of BMP expression is a common finding in ACC and we provide evidence that BMP dependent pathways might be involved in modulation of the malignant phenotype of this cancer.

OC4.4

Comparison of different test systems for the screening of inhibitors of CYP11B enzymes

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Subject
Inhibitors of CYP11B enzymes, particularly of aldosterone synthase, are currently under development as adrenostatic agents in heart failure or hyperaldosteronism, and for use in adrenal imaging. As CYP11B1 and CYP11B2 show high homology, analysis of specific binding to the respective human enzymes is of key importance.
Methods
Y1 cell lines stably expressing hsCYP11B1 or hsCYP11B2 were established. Primary cultures of porcine and human adrenocortical tissue were prepared. To assess the comparability of different systems NCI-h295 cells, transfected Y1 cells, and primary cultures were treated with different CYP11B inhibitors. Measurement of aldosterone and cortisol in the supernatant was performed.
Results
Potency as well as specificity of the tested inhibitors to CYP11B enzymes showed substantial differences in the respective test systems. The determined IC50 values of several inhibitors tested in porcine primary cultures were an order of magnitude higher compared to human primary cultures. In contrast, NCI-h295 and hsCYP11B1 and CYP11B2 expressing Y1 cells led to results similar to those from primary cultures of human adrenal glands. For example, IC_{50} values for etomidate for inhibition of Cyp11B1: porcine primary cultures 69.4±0.7 nmol/l; human primary cultures 5.6±1.5 nmol/l; NCI-h295 cells 4.7±1.5 nmol/l; Y1-HsCyp11B1 cell line 1.1±0.7 nmol/l.
Conclusion
Due to the poor availability of human adrenal glands for primary cultures as well as their limitations due to their continuous loss of activity, a comparable test-system for CYP11B inhibitors is strongly required. Due to the presence of both CYP11B enzymes within the same cell and potential interaction of steroidal intermediates, NCI-h295 cells are of limited value for selectivity testing. Y1 cell lines expressing human CYP11B1 and CYP11B2 enzymes, respectively, are suitable and easy to use systems for screening of inhibitors.

OC4.5

Comparison of 1-mg versus 8-mg dexamethasone suppression tests (DST) in patients with clinically inapparent adrenal adenoma.

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Recent guidelines recommend to use the 1-mg DST to screen for subclinical hypercortisolism assuming that a post-DST cortisol >5.0 μg/dl define this condition. However, several experts suggest a more sensitive cut-off at 1.8 μg/dl while others consider mandatory to confirm unsuppressibility with a 8-mg DST. A consecutive series of 64 patients (22 men, 42 women, aged 28–81 years) with clinically inapparent adrenal adenoma were studied between 2005 and 2007. The adrenal masses were discovered serendipitously during diagnostic work-up of non-adrenal diseases and none of the patients presented classic cushingoid features. All the adrenal masses displayed typical CT characteristics of adrenocortical adenoma. The endocrine work-up included: 1-mg DST, 24-h UFC, ACTH, cortisol, midnight serum and salivary cortisol, DHEAS, PRA, aldosterone, urinary fractionated metanephrines. Urinary fractionated metanephrines, PRA and aldosterone resulted in the normal range in all cases. Fifty-two patients (81.2%) did not suppress cortisol <1.8 μg/dl after 1-mg DST (additional alteration of HPA axis in 44.2%) and 11 patients (17.2%) did not suppress <5.0 μg/dl (additional alterations of HPA axis in 63.6%). A subgroup of 22 non-suppressor patients were further investigated with the 8-mg DST. Cortisol levels after 8-mg DST were surprisingly higher than 1-mg DST (4.9±2.2 vs 7.5±4.5 μg/dl, $P=0.09$). In only 4 patients (18.2%), cortisol levels were lower after 8-mg than 1-mg DST, being <1.8 μg/dl in 2 cases. Even in the 11 patients with cortisol >5.0 μg/dl following 1-mg DST, cortisol levels were higher after 8-mg DST (7.0±1.5 vs 9.6±4.2 μg/dl, $P=0.22$). These data support the view that secretory autonomy is a common feature of incidental adrenal adenomas. Cortisol is secreted without a complete restrain by pituitary feedback even when the degree of cortisol excess appears to be minimal. Interestingly, greater dexamethasone doses induce paradoxically higher cortisol levels than after 1-mg DST.

OC4.6

Isolation and characterization of cells coexpressing endothelial progenitors and parathyroid specific genes from human adult normal and tumoral parathyroids

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A peculiar characteristic of parathyroid tissue is the ability to spontaneously induce angiogenesis, to proliferate and to secrete PTH when autotransplanted in patients undergoing total parathyroidectomy. Since stem/progenitor cells have been involved in the process of tissue regeneration, we searched for putative parathyroid progenitors from human normal and tumoral parathyroids. By immunohistochemistry, FACS analysis and cell culture we identified parathyroid cells positive for the haematopoietic/endothelial marker CD34

which expressed surface antigens typical of endothelial progenitors, such as CD146 and CXCR4, but not the haematopoietic and mesenchymal markers, such as CD45, Thy-1/CD90, CD105 and CD117/c-kit. These cells were more abundant in tumoral than in normal parathyroids (4.4±1.2 and 2.2±0.9% respectively; $P=0.05$), without any difference in their immunophenotype except for the expression of nestin, a neural stem cell specific marker, which was almost exclusively restricted to the tumor-derived $CD34^+$ population. Purified $CD34^+$ cells, but not $CD34^-$ cells, proliferated, although at a low rate, and differentiated into mature endothelial cells. Finally, parathyroid specific markers, such as glial cell missing B, PTH and calcium sensing receptor, were detected at mRNA and/or protein level in a subset of $CD34^+$ cells. In conclusion, we identified cells which expressed both endothelial and parathyroid specific markers in human adult normal and tumoral parathyroids, providing a candidate for the putative parathyroid progenitors. Further studies are need to demonstrate the potential differentiation in multiple lineages and the self-renewal ability of these cells.

OC4.7

Molecular analysis of the calcium sensing receptor (CaSR) gene in 40 patients suspected to have familial hypocalciuric hypercalcemia (FHH)

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Neonatal severe hyperparathyroidism (NSHPTH) and FHH, usually defined as a ratio of calcium clearance/creatinin clearance <0.01 with normal kidney function and vitamin D status, are caused by respectively heterozygote and homozygote inactivating mutations of the *CaSR* gene. The aim of this study was to assess the interest of analyzing CaSR in hypercalcalcemic subjects suspected to have FHH.
Patients and methods
Forty hypercalcaemic subjects from 38 families were evaluated because of hypercalcaemia associated to relative hypocalciuria ($n=34$), familial hypercalcaemia ($n=12$) or post-surgical recurrence ($n=3$).
Results
Five subjects from 4 families (11.7% of index cases) had a CaSR gene mutation, 3 never described before (P666L, G94R, A295T, R648X). One patient presented an insertion in the intra-cellular domain of the CaSR protein of unknown signification. CaSR gene polymorphisms were identified in 31 patients (A986S:12, R990G:4, A986S+Q1011E: 2). Three patients had strictly normal CaSR gene. The phenotypic characteristics of patients showing mutations, polymorphisms or normal CaSR gene were not different except for a younger age in mutated patients ($P<0.05$). Among mutated patients, hypocalciuria was not always present. After exclusion of the NSPHPT, the ratio calcium clearance/creatinin clearance, did not appear to be a good diagnosis criterium for FFH, with a sensitivity of 60%, a specificity of 45%, a positive predictive value (PV) of 16% and a negative PV of 90%.
Conclusion
In concordance with recent data (Nissen *JCEM* 2007), these results suggest that a variety of biochemical phenotypes are linked with the genetic diagnosis of FFH. This could depend on the type of mutation but also on vitamin D status (Zajickova *JCEM* 2007). By contrast, a certain number of typical cases are not associated with CaSR gene abnormality, suggesting anomalies of another calcium receptor, of CaSR transduction protein, or antiCaSR antibodies (Makita *PNAS* 2007).

OC4.8

Incidental parathyroidectomy during thyroid surgery: outcome, follow-up and management of 231 patients treated between 1989 and 2007

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Background
Hypocalcemia is one of the most frequent complications after thyroid surgery. The aim of this study was to investigate the clinical relevance, outcome, follow-up and management of patients with incidental parathyroidectomy during thyroid surgery.
Subjects and methods
Two hundred and thirty-one patients (5.2%, 200 females and 31 males) of 4206 operated patients, which presented between 1989 and 2007 in our out-patient endocrine department, suffered from hypoparathyroidism and were retrospectively evaluated. Fifty-eight percent of these patients had undergone total thyroidectomy, 42% partial thyroidectomy. Preoperative diagnoses were: goiter with or without thyroid nodules 64%, Graves' disease 12%, thyroid cancer 20%.
PTH and serum calcium were assessed on the first visit and further on.
Results
Patients presented after a mean of 84±132 months after thyroid surgery. Clinical symptoms at the first visit were: paresthesia in 36% and cramps/tetany in 24%/13%. A total of 57% recieved no treatment – neither calcium nor vitamin D – at the first visit, 22% of patients received 1,25-hydroxyvitamine D3, 13% dihydrotachysterol, 8% vitamin D3 and 1% 1-alpha-hydroxyvitamie D3. PTH levels at the first visit under the different treatment regimens were 14.24±6 pg/ml, calcium levels 2.16±0.6 mmol/l.
At a second visit 4–12 months later symptoms had decreased to: paresthesia in 24% (at visit 4 (V4) after another year or more: 6%), cramps/tetany in 5%/2% (V4: 3/0%). Most patients received treatment with 1,25-hydroxyvitamine D3: 27% at visit 2 and 18% at V4. Calcium levels at visit 2 were: 2.18±0.51 and 2.17±0.21 mmol/l at visit 4.
Conclusions
Permanent hypoparathyroidism following incidental parathyroidectomy during thyroid surgery is a common but often disregarded complication – mainly in female patients. Patients after thyroid surgery should be regularly screened for clinical symptoms and low PTH- or calcium-levels. Treatment with 1,25-hydroxyvitamine D3 seems to be an appropriate therapy to maintain (near-) normal calcium levels and preserve patients from symptoms of hypocalcemia.

OC4.9

Teriparatide (TPTD) treatment followed by either zoledronic acid (ZOL) 5 mg once yearly or strontium ranelate (SR) 2 g daily: preliminary results from the ZOSTER-Study

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Background
TPTD treatment has been shown to reduce vertebral and non-vertebral fracture risk in postmenopausal osteoporosis efficiently. However, gains in bone mass achieved by this treatment are almost lost 2 years after cessation of treatment, unless consecutively followed by an antiresorptive treatment such as alendronate.
Subjects and methods
In the present prospective randomised open-labelled study in 52 pmp women, we investigated the effect of either 5 mg of ZOL once yearly versus 2 g of SR daily, following 18 months of TPTD treatment. Biochemical markers of bone turnover were determined before, and after 18 months of TPTD treatment. Furthermore, markers were obtained at months 3, 6, and 12 after initiation of treatment with either ZOL or SR.
Results

Table 1 Numbers represent mean absolute values and percentual change in serum bone resorption and formation markers.

	After 18 months	3 months		6 months		12 months	
	TPTD	ZOL	SR	ZOL	SR	ZOL	SR
OC (ng/ml)	60.2	16.2* (−73%)	30.1 (−51%)	16.8* (−72%)	26.0 (−58%)	18.4* (−69%)	27.9 (−55%)
CTX (ng/ml)	0.57	0.09* (−85%)	0.38 (−26%)	0.15* (−77%)	0.28 (−46%)	0.18* (−72%)	0.36 (−32%)
P1NP (ng/ml)	101.6	14.5* (−86%)	38.6 (−62%)	18.5* (−82%)	31.0 (−70%)	24.2* (−76%)	38.4 (−63%)
TRAP (U/l)	4.5	2.2* (−53%)	3.7 (−15%)	2.5* (−47%)	3.3 (−23%)	2.8* (−41%)	3.6 (−17%)

OC, Osteocalcin; CTX, beta CrossLaps; P1NP, type-I procollagen aminoterminal propeptide; TRAP, tartrate resistant acid phosphatase. (*$P<0.05$ for differences between ZOL- and SR-group; ANOVA).

Conclusion
Biochemical markers of both bone resorption and formation are significantly more suppressed after one year of treatment with a single dose of ZOL 5 mg as compared to one year of treatment with SR 2 g once daily. However, the significance of these findings on bone quality is currently unclear, since paired bone biopsies collected in this study have not yet been evaluated.

Reproduction–OC5

OC5.1

Female fertility defects and puberty delay in micelacking activin receptor-like kinase 7

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Members of the TGF-β superfamily of ligands signal through a combination of Type I and Type II receptors. Type I and Type II receptors have been related to various human diseases including developmental malformation, cancers and endocrine disorders. However, little is known about the function of ALK7, a Type I receptor expressed in prenatal and adult CNS and various endocrine tissues. We have generated a mice lacking ALK7, which are viable and fertile but display metabolic and reproductive phenotypes. In the present study, we have analyzed the expression of ALK7 in different reproductive organs, the onset of puberty, and fertility in female mice lacking the receptor. Vaginal estrus cycles were monitored from postnatal day 30 to postnatal day 60. In mice lacking ALK7, the first vaginal estrus is delayed compared to wild type females. Also, during this peripubertal period, estrus cyclicity in knockout mice is delayed and irregular. Interestingly, body weight is increased in knockout female mice during the peripubertal period, but not at any other age. Mice that lack the ALK7 receptor develop reproductive defects, including fewer litters and pups per litter during their fertile period. In addition, knockout mice have a marked delay in producing their first litter. Puberty delay and subfertility in the ALK7 knockout females suggest an important role for ALK7 in puberty onset and maintenance of the reproductive function.

OC5.2

The T222P mutation of the LGR8 gene is not causative for cryptorchidism

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Introduction
INSL3 and its receptor LGR8, are essential for the first phase of testicular descent. Homozygous loss of either of the two genes in mice leads to cryptorchidism. Even though mutations in both homologous human genes are not a common cause of cryptorchidism. To date, only one missense mutation at codon 222 (T222P) of the LGR8 gene has been proposed as causative mutation for cryptorchidism. This conclusion was based on both functional *in vitro* studies and the lack of mutation in a large group of controls. The geographic origin of the mutation carriers suggested a founder effect in the Mediterranean area.
Objectives
We sought to define the frequency of the T222P mutation in four different countries in order to assess whether the screening for this mutation could be of use as a diagnostic genetic test.
Materials and methods
A total of 822 subjects (359 with a history of cryptorchidism and 463 controls) from Italy, Spain, Hungary and Egypt were genotyped for the T222P mutation by direct sequencing.
Results
The phenotypic expression of the mutation included also normal testicular descent. The mutation frequency was not significantly different in cryptorchid patients versus non cryptorchid controls (3.6 vs 1.7%). No significant geographic differences were observed in mutation frequencies. The haplotype analysis allowed to predict three distinct haplotypes i.e. three possible mutation events.
Conclusions
Our results suggest that the T222P mutation cannot be considered neither causative nor susceptibility factor for cryptorchidism. A true causative mutation in the LGR8 gene remains still to be identified.

OC5.3

Prokineticin-2 and prokineticin receptor-2 gene analysis in men with Kallmann syndrome or normosmic hypogonadotropic hypogonadism

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Prokineticins (PK1 and PK2) are peptides regulating multiple biological processes through two G-protein coupled receptors, PK-R1 and PK-R2. PK2/PKR2 signalling is critical for neurogenesis of olfactory bulb and GnRH migration. Mutant mice lacking PKR2 have abnormal development of olfactory bulb and reproductive system atrophy, suggesting that these genes may be novel candidate for Kallmann syndrome (KS) in humans. Recently, mutations in PK2 and PKR2 genes have been found in near 10% of KS subjects.
Methods
We analyzed 38 men with idiopathic hypogonadotropic hypogonadism (IHH): 22 with associated anosmia/hyposmia (KS) and 16 with normosmic IHH (nIHH). PK2 and PKR2 coding exons were amplified by PCR on genomic DNA extracted from blood, deletion analysis and sequencing of obtained PCR fragments were performed.
Results
We found 4 mutations in the KS group: at exon 1 of PK2 in heterozygous state (Ile30Thr), and at exon 2 of PKR2 (Val274Asp homozygous, Arg268Cys and Leu173Arg heterozygous).
Discussion
We found a mutation of PK2 or its receptor R2 gene in 20% of KS and in none of nIHH and control DNAs. Two mutations we identified were novel (Ile30Thr on PK2, and Val274Asp on PKR2) and both at highly conserved residues of gene, providing important clues about the role of the wt residues in PK2 and PKR2 stability and function. Our findings confirm that alterations of the PK2–PKR2 pathway may be responsible of KS.

OC5.4

Molecular and functional characterization of BMP15 variants associated with secondary amenorrhea and premature ovarian failure (POF)

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BMP15 is an oocyte-derived growth factor belonging to TGF-β superfamily involved in follicular development as a critical regulator of granulosa cell processes. BMP15 gene maps at Xp11.2 and is expressed throughout folliculogenesis. BMP15 is translated as a pre-proprotein consisting of signal peptide, pro-region and mature peptide. The pro-region has an important role in the processing driving the pro-protein dimerization and secretion of active mature dimers. We report the genetic screening of 250 unrelated idiopathic POF women affected by primary or secondary amenorrhea. Six heterozygous BMP15 variations have been identified in association with secondary amenorrhea and POF. All novel alterations are non conservative and include a 3 bp insertion (262insLeu) and five missense substitutions in the proregion or signal sequence: S5R, R68W, R138H, L148P, A180T. Only the insertion was found in 100 control women with physiological menopause. To clarify the role of these variants, we characterized their molecular processing by Western blot and their biological activity by a luciferase-reporter assay using the COV434 granulosa cell line. We attest that 262insLeu is a polymorphic variant provided with normal processing and biological activity, consistent with its finding in controls. Deleterious effects *in vitro* were not detected also for A180T and S5R variants. In contrast, significant impairment of precursor processing and 5–20 fold reduction of mature BMP15 protein secretion were seen for the other mutations. Moreover, R68W, L148P and R138H biological activities were significantly impaired in comparison with the wild-type even if tested in co-transfection experiments which reproduce the heterozygous state seen *in vivo*. Our findings indicate that haploinsufficiency of BMP15 gene is associated with secondary amenorrhea and POF, and support the idea that BMP15 may be the first ovary determining gene on X chromosome whose haploinsufficiency may account for the ovarian defect in Turner syndrome.
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OC5.5

Prevalence of low testosterone levels in primary care in Germany: cross-sectional results from the DETECT study

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Background
Low testosterone levels in men occur with increasing age and are associated with increased morbidity, particularly metabolic syndrome, and mortality. As the prevalence of hypogonadal testosterone levels has not been assessed in the primary care setting in Europe, we aimed to investigate the prevalence of low testosterone levels in this setting, and the patient characteristics and comorbidities associated with it.
Methods
We measured testosterone in 2719 male primary care patients (age 58.7±13.4) from the DETECT study, a nationwide representative study of cardiovascular risk in Germany. Information on diseases, risk conditions and treatments was recorded by the primary care physicians. A large set of laboratory parameters was measured in a central laboratory. We calculated univariate and multivariate logistic regression models to assess the associations of low testosterone levels with different health and life style factors.
Results
A total of 19.3% of all men had hypogonadism as defined by testosterone levels <3.0 ng/dl. Stepwise logistic regression analysis revealed that obesity, metabolic syndrome, cancer, intake of six or more drugs, acute inflammation, and non-smoking were associated with hypogonadal testosterone levels. Higher age, liver diseases, and cancer were associated with very low testosterone levels (<1.0 ng/dl).
Conclusions
Hypogonadal testosterone levels are common in primary care, particularly in patients with the above conditions.

OC5.6

Effects of pregnancy on GH/IGF-1 concentrations in acromegalic women

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In normal woman, placental GH secretion increases during gestation and induces an increase of IGF-1 concentrations. In acromegalic women, increased pituitary GH secretion seems autonomous and IGF-1 increases in late stage of pregnancy related to placental GH. In a cohort of 46 women (mean age 31.7±4.5 years), acromegaly was due to micro ($n=7$) and macro ($n=39$) adenomas. Before the beginning of 59 pregnancies, women have been treated by transphenoidal surgery ($n=47$), pituitary radiotherapy ($n=2$) and medical therapy (dopamine agonists $n=25$, somatostatin analogs $n=14$), and GH/IGF-1 hypersecretion was controlled ($n=23$) and uncontrolled ($n=34$). A decrease of IGF-1 concentration was observed during the first (12/17), second (13/18) and third (6/9) trimester of gestation. In women with a decrease of IGF-1 concentration during the first trimester of gestation (before 588±207 ng/ml, first trimester 319±126 ng/ml, $P=0.002$), GH concentration was stable (10%), decreased (55%) and increased (35%). Therefore, the decrease of IGF-1 concentration during the first trimester of pregnancy is not correlated with changes of GH levels (before 5.8±4.2 ng/ml, first trimester 5.2±4.9 ng/ml). In such acromegalic women, the decrease of IGF-1 concentration may be related to increased estrogen levels resulting in a relative hepatic GH-resistance state. Moreover, pituitary GH secretion of some women may not be entirely autonomous and may be partly sensitive to negative feed back control of IGF-1. In conclusion, serum GH and IGF-1 concentrations show variable profiles during gestation of acromegalic women indicating that monitoring of GH/IGF-1 levels is not mandatory during uneventful pregnancy. On the other hand, decrease of IGF-1 level is possible during gestation implying that medical treatment could be discontinued during gestation of most women with acromegaly.

OC5.7

Hepatic steatosis is not a feature of young lean women with polycystic ovarian syndrome

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Background
Non-alcoholic fatty liver disease (NAFLD) is a very common liver disorder associated with insulin resistance and the metabolic syndrome. Insulin resistance is also a prominent feature even in lean women with the polycystic ovarian syndrome (PCOS). The objective of this study was to identify the presence of NAFLD in young lean women with PCOS.
Methods
Fifteen women with PCOS and 10 controls participated in the study.
Mean age was 25±4.65 and 24.5±5.94 years and mean body mass index was 21.7±2.5 and 23.5±3 kg/m^2 for the PCOS and the control group respectively. All participants had baseline biochemical, metabolic and hormonal profile and underwent a 75 g oral glucose tolerance test with measurements of glucose and insulin every 30 min for 2 h. The following indices of insulin resistance were calculated: GIR=fasting glucose/fasting insulin, AUC (area under the curve) GIR=AUC gluc/AUC insulin, MATSUDA=10 000/square root (fasting glucose×fasting insulin)×(mean glucose×mean insulin). Only women with PCOS underwent imaging studies with ultrasound and computed tomography of the liver. Fatty infiltration of the liver was considered as positive when the CT attenuation of the liver was at least 10 Housefield Units below the CT attenuation of the spleen.
Results
Women with PCOS had higher total testosterone (0.62±0.42 vs 0.36±0.08 ng/ml, $P=0.03$), free androgen index (7.4±6.4 vs 2.6±0.9, $P<0.05$) and indices of insulin resistance (GIR $P<0.05$, AUC INS $P<0.05$, AUC GIR $P<0.001$, MATSUDA $P<0.05$) compared to controls. Although there was no evidence of NAFLD in imaging studies, a significant negative correlation between mean hepatic attenuation and waist to hip ratio ratio was found ($r=-0.55$, $P=0.034$).
Conclusions
Young lean women with PCOS do not have NAFLD besides insulin resistance. Studies with more prolonged follow-up are required to determine the evolution of hepatic changes in such patients.

OC5.8

The effect of estrogens plus cyproterone acetate or orchiectomy on serum insulin like factor 3 (INSL3) levels in transsexual men

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The regulation of insulin-like factor 3 (INSL3) secretion from Leydig cells is still incompletely clarified. In this study we measured INSL3 serum levels in transsexual men under estrogen (E) and antiandrogen therapy or after orchiectomy. The effects of gonadotropins on testicular secretion *in vitro* was also evaluated.
Methods
Blood samples were obtained from 14 transsexual men under long-term E ($n=5$) or E plus cyproterone acetate (E+CPA, $n=9$) therapy. Samples were also drawn in 5 TS before and after 1, 3, 7 and 30 days from orchiectomy for gender change. Normal control plasma level was evaluated on ten adult men (NC). Short-term testicular tissue cultures were set-up and INSL3 secretion evaluated in medium obtained basally or after 72-h treatment with hCG, rFSH or both. INSL3 was measured in serum or medium using a commercial kit (Phoenix, Germany).
Results
Serum INSL3 resulted significantly reduced in TS assuming E+CPA (175±17 vs both TS E treated 346±57 and NC.711±76 pg/ml, $P<0.0001$). In all TS treated with E+CPA LH values were undetectable. INSL3 levels decreased immediately after orchiectomy (baseline 267±80, 1st day 89±5.8) remaining lower on 30th day. hCG induced a sharp INSL3 increase *in vitro* (+107%), whereas rFSH did not affect NSL3 secretion.

Conclusion
Reduced serum LH and INSL3 levels in E+CPA treated TS and hCG/LH stimulation of INSL3 release from testicular tissue *in vitro* confirm that LH plays a major role in regulating testicular secretion. Although detectable serum INSL3 levels in orchiectomized TS suggest an extra gonadal surge, marked decline immediately after surgery indicates that testes are the main site of INSL3 production in men.

OC5.9

Cognitive disabilities in the novel object task of male mice carrying a supernumerary X chromosome (41, XX*Y)

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Introduction
Several numerical chromosome abberations are known in men. Of those the karyotype XXY (Klinefelter syndrome KS) is the most common chromosomal disorder with a prevalence of about one in 500 live-born males. KS is associated with hypogonadism and is suspected to cause variable physical and cognitive abnormalities. As a supernumerary X chromosome is also associated with infertility, sound animal models for KS are difficult to obtain.
Methods
Male mice with two X chromosomes (XX^{*Y}) were derived from fathers carrying a structurally rearranged Y chromosome (Y*) that resulted in close attachment of a part of the Y chromosome to one X. These animals seem to mimic physiological features of the human KS. The aim of this study was a behavioral characterization of this mouse model. Therefore, 15 XX^{*Y} males and 15 XY* controls were subjected to a battery of behavioral tests including a general health check, analysis of spontaneous exploration and locomotor activity, measures for anxiety related behavior and the novel object task to test memory performance.
Results and discussion
All mice appeared healthy. XX^{*Y} mice did not differ from their wildtype littermates with respect to locomotion, exploration and anxiety related behavior. XX^{*Y} male mice, however exhibited no significant learning performance in contrast to wildtype XY* males which readily learned the task. These findings support the idea that the presence of a supernumerary X in male mice influences cognitive abilities and that the 41, XX^{*Y} mouse model can also be utilized to study X chromosomal imbalance and cognition experimentally.

Metabolism and cardiovascular–OC6

OC6.1

The signaling profile of recombinant human orexin-2 receptor (OX2R)

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Orexin-A and orexin-B orchestrate their diverse central and peripheral effects via two G-protein coupled receptors, orexin-1 receptor (OX1R) and OX2R, which activate multiple G-proteins. In many tissues, orexins activate MAPKs, however, the mechanism by which OX2R alone mediates MAPK activation is not understood. We studied the intracellular signaling mechanisms involved in OX2R-mediated $ERK_{1/2}$ and p38 MAPK activation. In HEK-293 cells stably over-expressing recombinant human OX2R, orexin-A/B resulted in a rapid, dose and time dependent increase in activation of $ERK_{1/2}$ and p38 MAPK, with maximal activation at 10 min for $ERK_{1/2}$ and 30 min for p38 MAPK. We used dominant-negative G-proteins and selective inhibitors of intracellular signaling cascades and pertussis toxin, and determined that orexin-A and orexin-B induced $ERK_{1/2}$ and p38 MAPK activation through multiple G-proteins and different intracellular signaling pathways. $ERK_{1/2}$ activation involves Gq/phospholipase-C (PLC)/protein kinase-C (PKC), Gs/adenylyl cyclase (AC)/cAMP/protein kinase A (PKA) and Gi cascades; however, the Gq/PLC/PKC pathway, as well as PKA is not required for OX2R-mediated p38 MAPK activation. Interestingly, as compared to orexin-A, the Gq/PLC/PKC pathway plays a predominant role in orexin-B-induced $ERK_{1/2}$ activation. In conclusion, this is the first comprehensive signalling study of the OX2R recombinant receptor, showing $ERK_{1/2}$ and p38 MAPK activation are regulated by differential signaling pathways in HEK-293 cells, and that the $ERK_{1/2}$ activation is severely affected by naturally occurring mutants associated with narcolepsy. Moreover, it is evident that the human OX2R has ligand specific effects, with orexin-B being more potent in a transfected system. This distinct modulation of the MAPKs through OX2R may contribute to the regulation of diverse biological actions of orexins.

OC6.2

Human adipocytes secrete new cardiodepressant factors: a direct link between obesity and heart failure

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Obesity has long been recognized as an independent risk factor in heart failure. The mechanisms proposed to explain this correlation include hemodynamic changes secondary to obesity, leading to left ventricular remodeling and systolic dysfunction, and cardiomyocyte apoptosis induced by increased lipid accumulation into cardiomyocytes. Adipose tissue is an endocrine organ that secretes a wide variety of bioactive factors, which seem to mediate communication between adipose tissue and other organs such as muscle, liver, pancreas and brain. In this study, we investigated whether human adipose tissue may directly affect heart contractile function by secreting cardioactive substances. We cultivated adipocytes obtained from human white adipose tissue, and treated isolated rat adult cardiomyocytes in cell culture as well as isolated rat heart in Langendorff system with adipocyte-conditioned or control medium. We observed that human adipocytes release factors that strongly and acutely suppress contraction of electrically paced adult cardiomyocytes by attenuating intracellular Ca^{2+} levels. These adipocyte-derived cardiodepressant factors could be completely blunted by heating and by trypsin-treatment, indicating the involvement of a protein. Using filtration devices, the cardiodepressant factors could be removed by 10 kDa cutoff filtration, but remained after 30 kDa cutoff filtration, defining the molecular weight of the protein between 10 and 30 kDa. Likewise, isolated rat heart perfused with adipocyte-conditioned medium revealed a reversible strong decrease of force generation and of coronary flow due to contraction of the coronary vessels. In conclusion, our findings revealed a hitherto unknown acute cardiodepressant effect of adipocyte-derived factors directly on cardiomyocytes by suppressing intracellular Ca^{2+} and indirectly by reducing coronary flow, thus suggesting a direct role of adipose tissue in the pathogenesis of heart failure in obese patients.

OC6.3

Visfatin induces MMP-2/9 activation in human vascular endothelial cells *via* nuclear factor-κB

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Aim
Cardiovascular disease is more common in individuals with diabetes mellitus and obesity. Visfatin is elevated in obesity and T2DM; and thought to be an inflammatory mediator within atherosclerotic lesions inducing gelatinase activity, contributing to plaque destabilisation. We investigated the activation of NF-κB, a well-known pro-inflammatory transcription factor, by visfatin, in Human Endothelial Cells (EC).
Research design and methods
We stably transfected EAHy926 (human) cells with a *cis*-reporter pNF-κB-Luc plasmid containing luciferase reporter gene linked to five repeats of NF-κB binding sites. Using luciferase reporter assay, quantitative PCR, western blotting and gelatin zymography, we studied the involvement of NF-κB signalling in gelatinase mediated vascular inflammation by visfatin; employing the NF-κB inhibitor, BAY 11-7085.
Results
Visfatin induced a significant dose dependent increase in NF-κB mediated transcriptional activity ($P<0.001$), with a comparable potency to TNFα, a robust inducer of NF-κB activity. Also, when transfected ECs were pre-incubated with visfatin for 16 h, followed by TNFα stimulation for 2 h revealed a significant inhibition of TNFα induced NF-κB activity ($P<0.001$), suggesting receptor desensitisation. Furthermore the NF-κB inhibitor, BAY 11-7085, significantly down-regulated visfatin induced MMP-2 and MMP-9 expressions at mRNA, protein and zymogen levels.
Conclusions
We were able to demonstrate that visfatin is a profound stimulator of NF-κB transcriptional activity in human ECs, also the crucial involvement of NF-κB signalling in visfatin induced activation of gelatinases-MMP-2/9. Moreover, the hypo responsiveness of NF-κB mediated transcriptional activity induced by visfatin is of importance since pro inflammatory cytokine 'overload' exists in obesity and T2DM suggesting an important role of visfatin in the pathogenesis of vascular inflammation.
In summary, our finding reveals a new insight into visfatin's diverse roles in dysmetabolic states and reaffirms the emerging roles of adipokines as mediators of inflammatory response.

OC6.4

Lipolysis in subcutaneous tissue after intranasal administration of ACTH(4-10) is associated with an increased baroreceptor reflex sensitivity.

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The melanocortin system plays a significant role in the hypothalamic regulation of body weight and energy expenditure. Prolonged central administration of the melanocortin receptor 4 (MC-4) agonist ACTH(4-10) reduces body weight in animals and humans by increasing energy expenditure and by reducing food intake. The aim of this study was to investigate the effect of intranasally administered alpha-MSH on local lipolysis in subcutaneous adipose tissue and on sympathetic nerve activity (SNA) to the muscle (MSNA). After an overnight fast ten healthy and normal weight volunteers received either 10 mg ACTH(4-10) or placebo intranasally in a double-blind randomized fashion on two consecutive days. Microdialysis for the detection of glycerol, glucose and lactate was performed in abdominal subcutaneous adipose tissue; simultaneously we recorded SNA to the lateral femoral nerve. After intranasal application of 10 mg ACTH(4-10) glycerol in adipose tissue increased and was significantly higher after 45 min when compared to placebo (Δbaseline = $53.4 \pm 19.3\%$ for ACTH(4-10), $22.2 \pm 14.1\%$ for placebo, $P < 0.05$). Interstitial lactate and glucose did not show any significant changes. Basal MSNA was not different after ACTH(4-10) or placebo, however, intravenous nitroprusside increased burst frequency by $569 \pm 69\%$ after ACTH(4-10) treatment but only by $315 \pm 64\%$ after placebo. This difference was statistically significant ($P = 0.012$). Previous experiments in humans have shown that intranasal administration of ACTH(4-10) yields sufficient concentrations in the cerebrospinal fluid. In our experiments, intranasally (centrally) administered ACTH(4-10) stimulates lipolysis in peripheral organs like adipose tissue. This central effect of the MC-4 agonist might be mediated through autonomous nerves since lipolysis was associated with an increase of baroreceptor reflex sensitivity which in turn could reflect an activation of the central sympathetic nervous system.

OC6.5

Association of the SHBG gene promoter polymorphism with early markers of atherosclerosis in apparently healthy women

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Objective
Androgen may be detrimental in the development of cardiovascular disease in women. We investigated possible associations between the (TAAAA)*n* polymorphism of sex hormone binding globulin (SHBG) gene promoter, which influences transcriptional efficiency of the SHBG gene and thus the tissue androgen availability and early markers of atherosclerosis in apparently healthy women.
Design and methods
In this prospective clinical study, 153 consecutive women (mean age 43.9 ± 9 years, 66 of whom postmenopausal, without known diabetes, hypertension, cardiovascular disease), visiting our internal medicine outpatients were examined for unrecognised features of the metabolic syndrome. Endothelium dependent vasodilatation (FMD), an early marker of atherosclerosis, and intima media thickness (IMT) of the common carotid artery were recorded. According to the number of SHBG gene promoter repeats patients were classified as short (≤ 7), medium ($=8$) and long repeat (≥ 9) allele groups.
Results
There was a significant negative correlation between FMD and the length of the two polymorphic alleles that each woman carried ($P = 0.032$ and $P = 0.01$, for the shorter and longer allele, respectively). IMT of the common carotid had a positive correlation with the length of the shorter allele ($P = 0.015$). Women carriers of two long alleles had increased IMT ($P = 0.037$, Anova). These associations were independent of BMI, abdominal obesity and HOMA insulin resistance index (step multivariate analysis, $P = 0.001$). Mean SHBG levels tended to be higher in women carrying the longer alleles ($P = 0.06$). Finally in the subgroup of premenopausal women there was a significant positive correlation between SHBG levels and FMD ($P = 0.037$).
Conclusions
Longer (TAAAA)*n* repeats in the SHBG gene promoter are associated with impaired FMD and increased carotid artery IMT, both of which are early markers of atherosclerosis. This association may reflect the life-long tissue exposure to higher free androgens and indirectly supports the view that androgenic exposure may have adverse cardiovascular effects in women.

OC6.6

Androgens increase blood pressure in orchiectomized male Wistar rats similar to high salt diet

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Introduction
With the onset of puberty blood pressure increases more in men than in women while boys and girls do not show any gender differences in blood pressure. Androgens are known to play an important role in renal tubular epithelial cell growth, hypertrophy and erythropoetin production and may be important determinants of sex-specific differences in blood pressure. However, the exact mechanisms are not clear yet.
Methods
Male Wistar rats aged 8–10 weeks were orchiectomized and put on a low- (diet 0.03% NaCl + tap water) or high-salt diet (diet 4% NaCl + trinking water 0.09% NaCl) over 5 weeks. In addition they received either placebo, testosterone (1 mg/animal) or 5alpha-dihydrotestosterone (1 mg/animal) as daily s.c. injection over 16 days (each group $n = 6$). In additional experiments, rats were treated with mineralocorticoid antagonist spironolactone (50 mg/kg weight per day) or androgen receptor antagonist flutamide (30 mg/kg weight per day). Blood pressure was measured non-invasively using the tail-cuff method. Twenty-four hours urine samples were collected in metabolic cages and blood and organs were secured after decapitation.
Results
Blood pressure significantly increased and aldosterone serum concentrations decreased due to high salt diet. This effect could be abolished by spironolactone as well as by flutamide. Testosterone and 5alpha-dihydrotestosterone significantly increased blood pressure in rats on a low salt diet. This effect could be diminished by flutamide and by spironolactone. The highly elevated aldosterone levels in rats on low salt diet with spironolactone or flutamide treatment were reduced by androgen treatment.
Conclusions
These results highlight the possible interaction of androgens with the mineralocorticoid pathway, probably via an androgen effect on the renal epithelial sodium channel (ENaC). Our data might give new insight into mechanisms responsible for the reported gender differences in blood pressure.

OC6.7

The mineralocorticoid receptor induces pro-inflammatory effects in white adipocytes and is essential for adipogenesis.

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Selective enhancement of glucocorticoid action in adipose tissue has been shown to induce characteristic features of the metabolic syndrome. Adipocyte-derived factors provide a link between altered adipose tissue mass and function and cardiovascular disease. However, key mechanisms controlling these pathophysiological alterations in adipose tissue are poorly understood. Recently, both, the glucocorticoid (GR) and the mineralocorticoid receptor (MR), have been shown to mediate glucocorticoid action in adipose tissue. We investigated the role of the mineralocorticoid receptor in controlling an inflammatory adipokine response and adipocyte differentiation. Selective GR stimulation of differentiated white adipocytes with dexamethasone dose-dependently inhibited gene expression of interleukin-6 (IL-6) and monocyte chemoattractant protein-1 (MCP-1) by 60 and 90%, respectively. By contrast, selective MR stimulation with aldosterone significantly induced the expression of these pro-inflammatory adipokines

by 50%. Furthermore, an acute knock-down of the GR in fully differentiated white adipocytes and subsequent corticosterone exposure strongly increased the mRNA expression of IL-6 and MCP-1 by 90 and 430%, respectively. Finally, novel adipose cell lines from MR- and GR-knock-out mice were generated. Whereas, preadipocytes from GR-knock-out mice showed mildly impaired accumulation of lipid droplets as compared to the wild-type control lines, MR-knock-out preadipocytes completely failed to accumulate lipid droplets. Taken together, our results dissect selective and opposing effects of the adipose MR and GR. The MR may play a role in inducing inflammatory adipocyte responses and appears essential in controlling adipocyte differentiation. Selective targeting of corticosteroid receptors may represent new options for the prevention and treatment of the metabolic syndrome and its cardiovascular complications.

OC6.8

Chronic prostaglandin excess results in adipose tissue wasting through increased substrate utilization

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Disruption of the balance in energy homeostasis and substrate metabolism can lead to obesity and the metabolic syndrome on one hand and body wasting in cachexia associated with cancer and other chronic diseases on the other hand. Indirect evidence suggests that prostaglandins (PGs), products of the cyclo-oxygenase (COX) pathway, are involved in the regulation of metabolic processes under physiological conditions or during disease, in particular cancer cachexia. However, the direct *in vivo* effects of PG action on peripheral tissues and the consequences for energy homeostasis have not been elucidated. To examine the effect of PG excess as a single factor on metabolism we analyzed the phenotype of transgenic mice over-expressing COX-2 locally in defined epithelia. These mice displayed increased PG E2 and F2alpha plasma levels, whereas no systemic inflammation could be detected. This was associated with severe wasting of abdominal adipose tissue, a process which developed with age and could be reversed by treatment with a selective COX-2 inhibitor. Interestingly, K5COX2 mice showed improved glucose tolerance and normal insulin sensitivity. Furthermore, the findings that K5COX2 mice were hyperphagic with elevated body temperature and reduced circulating free fatty acid levels suggest that PG-mediated adipose tissue wasting is a consequence of increased substrate utilization coupled to energy dissipation. Phenotypic analysis of adipose tissue in K5COX2 mice for the dissection of the underlying molecular mechanisms is currently underway. Taken together, our results highlight a previously unrecognized ability of PGs to modulate adipose tissue maintenance and energy expenditure *in vivo*. Modulation of PG-controlled pathways of metabolic regulation could represent an attractive approach applicable not only to the treatment of cachexia symptoms but also to the control of obesity.

OC6.9

The histone deacetylase inhibitor valproic acid (VPA) confers an estrogen-sensitive 'phenotype' to estrogen receptor-negative breast cancer cells MDA-MB 231

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Breast cancer cells may present a number of resistance mechanisms to conventional therapy, and therefore new treatments are under evaluation. The histone deacetylase inhibitor (HDI) valproic acid (VPA) is of particular interest, since having been used for a long time in neurological patients its side-effects and tolerability are well known. We have recently demonstrated that VPA is a powerful antiproliferative agent in estrogen-sensitive breast cancer cells. Unfortunately, its ability to arrest cell growth in estrogen-resistant breast cancer cells is poor. In the present study, we evaluated the effect of VPA on the expression of estrogen receptor alpha (ER alpha) and the estrogen-sensitivity of ER-negative breast cancer cells, MDA-MB 231. VPA induced the expression of ER alpha; VPA-treated cells were then exposed to 10 nM estradiol and some estrogen-regulated genes were evaluated with real time-PCR. After VPA treatment, estradiol up-regulated progesterone receptor (PR), pS2, and AREG, and down-regulated ER alpha itself, giving to MDA-MB 231 cells a behaviour similar to that of estrogen-sensitive cells. In order to clarify that the ER alpha induced by VPA in these cells was a fully functioning receptor, a transcription assay using as reporter the luciferase gene was performed. MDA-MB 231 cells, transfected with ERE-tk-LUC, were first treated with VPA and then exposed to estradiol, before evaluating luciferase activity. While in MDA-MB 231 cells in basal condition estradiol was not able to stimulate any luciferae activity, the answer of VPA-treated cells to estradiol was similar to that of estrogen-sensitive cells. In conclusion, the HDI VPA is able to induce ER alpha expression in ER-negative cells, giving them a completely estrogen-sensitive 'phenotype'. The possibility to restore estrogen-sensitivity in ER-negative breast cancer cells thus open up the chance to use anti-estrogen therapy even in this tumour type.

Poster Presentations

Adrenal

P1

Finasteride cream and idiopathic hirsutism

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Introduction
Hirsutism is the presence of excess terminal hair in women with a male pattern. Most cases of hirsutism are secondary to other disease but some of cases are idiopathic. The most accepted hypothesis for the development of hirsutism is increased 5-α reductase activity in skin. Based on this hypothesis, we have launched this study to evaluate the effects of Finasteride cream (a 5-α reductase inhibitor) on idiopathic hirsutism.
Methods
Forty cases of idiopathic hirsutism, have received 0.2% Finasteride cream twice a day for 6 months on their chins. Mean thickness of three hair samples for each patients were measured before therapy and after 6 months. Ferryman – gallwey score for the chin area was also defined.
Results
Mean hair thickness was decreased from 1.4 ± 0.89 μm to 0.96 ± 0.83 μm ($P < 0.001$). Mean Ferryman – Gallwey score was also decreased from 3.2 ± 0.41 to 2.2 ± 0.76 ($P < 0.001$). Acne was reported by 8 patients (20%) during the therapy. There were no other side effects.
Conclusion
Finasteride cream is an efficient and harmless therapy in patients with idiopathic hirsutism. Breast cancer cells may present a number of resistance mechanisms to conventional therapy, and therefore new treatments are under evaluation. The histone deacetylase inhibitor (HDI) valproic acid (VPA) is of particular interest, since having been used for a long time in neurological patients its side-effects and tolerability are well known. We have recently.

P2

Male sexual hormone effects on the adrenal cortex activity in a Saharan desert rodent, *Psammomys obesus* (Cretzschmar, 1828)

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The effect of gonadectomy and gonadal hormone replacement on the adrenal cortex activity was studied in the adult male sand rat *Psammomys obesus*, a diurnal gerbillid rodent living in Beni-Abbes area, in the Algerian Sahara desert (30°7 N, 2°10 W). Animals freshly captured were orchiectomized during the breeding season and some animals received testosterone oenanthate (75 μg per 40 μl olive's oil) twice a day during 7 days, 3 weeks after gonadectomy.
In the sand rat *Psammomys obesus*, orchiectomy increased the adrenal cortex weight (31.5%) and the adrenal content of RNA, DNA, and protein synthesis by respectively 45.1%, 46% and 32.1%. A highly significant increase in cholesterol (50.4%) and total lipids (71.5%) adrenal cortex content was observed after castration. Glucose and total carbohydrate content markedly increased by respectively 65.9% and 62.3%. Plasma cortisol decreased by −81.8% when plasma LH concentrations increased significantly in castrated by 98.9%.
The adrenal cortex apoptosis analysed by *in situ* end labelling of fragmented apoptotic nuclei DNA (TUNEL) showed that the highest apoptotic index was detected in the most inner zones of the adrenal cortex mainly in the zona reticularis. Nevertheless, androgen deprivation induced an increase in the labelling index in the zona fasciculata, with a decrease in the zona reticularis. The staining intensity in the zona glomerulosa was similar to that observed in non-castrated animals.
Testosterone oenanthate administration to castrated animals restored most of the investigated adrenal cortex content and the zonation of apoptotic cells changed to the status of intact sand rat.
It could be concluded that androgens act as regulatory factors for the programmed cell death and may be involved in the control of the mitotic activity and the turnover of adrenal cortex cells.

P3

Frequency of adrenal tumor in Japanese patients with type 2 diabetes mellitus

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Introduction
It is known that many patients with adrenal incidentaloma display altered glucose tolerance. However, to our knowledge, we have no reports of the frequency of adrenal tumor in Japanese type 2 diabetic patients. We investigated the frequency of adrenal tumor in these patients.
Subjects
We evaluated the presence of adrenal tumor in 245 Japanese type 2 diabetic patients (mean age 60.8 ± 13.4 years). The patients who were diagnosed malignant tumor and adrenal tumor previously were excluded.
Results
Nineteen patients (7.8%; mean age 62.0 ± 13.6) had adrenal mass determined by abdominal CT scan (Bilateral, right and left were 4, 8 and 7, respectively). The mean size was 14.1 ± 10.0 mm (maximum and minimum size were 55.0 and 1.0 mm, respectively). The properties of all adrenal masses were homogenous, and no cysts and calcification were observed. Measurement of plasma ACTH, renin activity, cortisol, aldosterone, DHEA-S and catecholamines, and 1 mg overnight dexamethasone suppression test were performed for diagnosis of functional adenoma. These adrenal masses were described as subclinical Cushing syndrome (SCS) and primary aldosteronism (PA) with SCS were 1 case for each, and 2 cases were PA. Fifteen were non-functioning tumor.
Discussion
It is reported that the frequency of adrenal incidentaloma in normal subjects was 3–4%. In our results, a relatively high prevalence, 7.8%, of adrenal tumor was observed in Japanese type 2 diabetic patients. On the other hand, the frequency of functional adrenal adenoma in diabetic patients was ∼25% which was similar those in normal subjects.
Conclusion
Although further studies are needed to evaluate the frequency of adrenal tumor in type 2 diabetic patients, a relatively high prevalence of adrenal tumor was found in our study. Therefore our data suggest that adrenal tumor should be evaluated positively in type 2 diabetic patients.

P4

Might the response of cortisol to oral glucose tolerance test be used for the differential diagnosis of Cushing's syndrome and pseudo-Cushing's states?

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The pseudo-Cushing states (PCS) include several clinical conditions such as obesity, polycystic ovary syndrome, depression and alcoholism, characterized by physical features and abnormalities of hypothalamus–pituitary–adrenal axis similar to Cushing's syndrome (CS). The differential diagnosis between CS and PCS is often complicated and requires several hormonal tests. The aim of this study is to evaluate the diagnostic accuracy of the cortisol response to the oral glucose tolerance test (OGTT) as a screening test to distinguish patients with PCS and CS. Twenty-six patients with CS (19 females, 7 males, 18–64 years) and 26 patients with PCS (21 females, 5 males; 18–63 years) entered the study. The clinical diagnosis of CS and PC were performed on the basis of urinary cortisol, serum cortisol circadian rhythm, low dose dexamethasone suppression test (LDDST), desmopressin test and/or CRH test after LDDST. The clinical diagnosis of PCS was confirmed by the clinical follow-up of the patients, which did not develop CS during the following 5 years. All patients were submitted to OGTT: serum cortisol levels were evaluated every 30 min for 2 h. Basal serum cortisol levels were significantly higher in CD than PCS (207.3 ± 49.9 vs 166.3 ± 56.5 ng/ml $P < 0.001$). Cortisol nadir was significantly higher (157.9 ± 49.2 vs 67.6 ± 28.9 ng/ml $P < 0.001$) whereas cortisol decrease percentage (22.9 ± 19.5 vs 57.6 ± 15.8% $P < 0.001$) was significantly lower in patients with CS than in

patients with PCS. Moreover, ROC analysis showed that a cut-off of cortisol nadir of 94.4 ng/ml was able to differentiate PCS from CD with 92% sensitivity and 89% specificity, and a cut-off of 60' after-OGTT-cortisol of 147.5 ng/ml was able to differentiate PCS from CD with 92% sensitivity and 92% specificity. These preliminary results suggest that OGTT might be considered as a valid screening test in the differential diagnosis of CS and PCS, taking into consideration that it is a simple test commonly used in the clinical practise to evaluate the glucose tolerance in patients with hypercortisolism, and with the only limitation for patients with diabetes mellitus. However, an extension of this study to a larger number of patients and the comparison of the diagnostic accuracy of OGTT and classical tests are mandatory to draw definitive conclusion of the usefulness of this test in the differential diagnosis of CS and PCS.

P5

Modulation of proteomic profile in h295r adrenocortical cell line induced by mitotane

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Background
Mitotane, 1,1-dichloro-2-(*o* chlorophenyl) -2- (*p*-chloro phenyl) ethane (*o*,*p*′-DDD) is a compound which represents the effective agent in the treatment of the adrenocortical carcinoma (ACC), able to block cortisol synthesis. Nevertheless the biological mechanism induced by this treatment in this cancer remains unknown. Recently, there has been a strong interest in applying proteomics to foster a better understanding of disease processes, mechanism of action and new pharmacological targets of drugs.
Aim
In this study, we describe the effects of mitotane on growth, steroidogenesis, cell cycle and proteomic profile on H295R cells, a characteristic model of ACC able to produce all the adrenocortical steroids, either in total cell extract or in mitochondria-enriched fraction after drug treatment.
Materials and methods
H295-R cells were treated with *o*,*p*′-DDD 10–5 M final concentration. The progesterone, testosterone, cortisol and aldosterone levels in the culture medium were determined by electrochemiluminescence (ECLIA) and immunoenzymatic assay. Flow cytometry cell cycle analysis was evaluated using FACScan cytofluorimeter. Total protein extracts and mithocondria-enriched fraction were employed on two-dimensional gel electrophoresis. Spots of interest were identified by peptide mass fingerprint on a Voyager-DE MALDI-ToF mass spectrometer.
Results
We confirmed that mitotane inhibited steroid secretion (Fig. 1) but not significantly influence cell growth (Fig. 2) and not induce perturbation of cell cycle (Fig. 3). Proteomic approach allowed us to identify a total of 18 proteins showing the characteristic expression changes in adrenocortical H295R cells treated with mitotane (Fig. 4). The proteins involved in drug response can be divided in different functional classes including: energetic metabolism, stress response, cytoskeleton structure and tumorigenesis (Fig. 5–6). In particular, a perturbation of the electron transport toward the steroidogenic cytochrome P450 enzyme systems was evidenced.

P6

Pheochromocytoma of the second adrenal gland 17 years after first adrenalectomy in PPS: case report

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Introduction
The mutation of SDHB gene is associated with familial pheochromocytoma-paraganglioma syndrome (PPS). SDHB gene is located in locus 1p23–25 and encodes a subunit B of succinate dehydrogenase, which plays a key role in the respiratory chain and Krebs cycle.
Pheochromocytoma in PPS usually produces norepinephrin. Even long term remission do not exclude relapse of the disease.
We present a case of bilateral adrenal pheochromocytoma due to familial pheochromocytoma-paraganglioma syndrome associated with SDHB mutation.
Case report
At the patient's age of 18 the first diagnosis of pheochromocytoma associated with typical clinical symptoms was made and followed by uncomplicated left adrenal gland tumor enucleation.
Seventeen years later during a follow up examination the right adrenal gland pheochromocytoma was suspected. The clinical manifestation, MIBG scintigraphy and metanephrine and katecholamine level results confirmed the presence of pheochromocytoma. Right adrenalectomy was performed without complication. The histopathological exam again revealed pheochromocytoma.
One year later the abnormal level of katecholamine and metanephrine in urine collection was revealed followed by raised tracer uptake in MIBG scintigraphy within left adrenal gland. Somatostatin analogues scintigraphy did not confirmed the lesion.
Due to the medical history of the patient left adrenalectomy was performed. The histopathological examination reveal normal adrenal gland tissue.
At the same time genetic testing (PCR) revealed the mutation of SDHB gene. The other members of the patient's family were healthy without presence of SDHB mutation.
By now the katecholamine and metanephrine concentration in urine collection and MIBG scintigraphy are normal.
Conclusion
The biochemical and imaging follow up should be perform for an extend period of time after surgery in a case of SDHB mutation. MIBG should be done in case of abnormal biochemical results. Genetic testing should be perform in any case of pheochromocytoma relapse, especially among young patients. The patient's family members should undergo genetic testing as well.

P7

Health related quality of life differs between three replacement therapies in adrenal insufficiency

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Objective
There is evidence that current replacement regimens fail to restore well-being in patients with adrenal insufficiency (AI). No data is available on the effect of different therapeutical regimes (hydrocortisone, prednisolone, cortisone acetate) on the quality of life in these patients.
Methods
About 883 patients with adrenal insufficiency were contacted, 526 patients participated (60%) and received a disease specific questionnaire and three standardized questionnaires (SF36, GBB24 and HADS). Reference data for SF-36 scores were obtained from the German National Health Survey comprising a representative random sample of 7124 subjects from the German population aged 18–79 years. Reference data for the GBB-24 ($n=2076$) and HADS ($n=2081$) were obtained from surveys performed by Brahler and colleagues. Finally, 428 patients (232 primary AI, 196 secondary AI) were analyzed regarding their glucocorticoid replacement therapies.
Results
Health related quality of life was impaired in both primary and secondary AI compared to age- and sex-matched controls. No significant differences in symptoms and complaints assessed by GBB24 were seen between hydrocortisone ($n=347$), prednisolone ($n=62$), and cortisone acetate ($n=19$) treatment. Anxiety and depression scores assessed by HADS indicated no significant differences between the treatments in all patients. However, the depression score was significantly ($P<0.05$) lower in patients with primary AI on prednisolone compared to hydrocortisone therapy. Symptoms and complaints assessed by SF36 did not show significant differences between the treatments in all patients. But in patients with primary AI, the score of bodily pain was significantly lower ($P<0.01$) in patients on prednisolone compared to hydrocortisone or cortisone acetate therapy.
Conclusion
Health related quality of life is impaired in patients with primary or secondary AI. Different glucocorticoid replacement therapies did not show differences in quality of life except in patients with primary AI regarding depression and bodily pain. These results suggest a need for improved glucocorticoid replacement strategies.

P8

Differences in quality of life between twice and thrice daily application of hydrocortisone in adrenal insufficiency

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Objective
There is evidence that current replacement regimens fail to restore well-being in patients with adrenal insufficiency. No data is available on the effect of twice or thrice daily administration of hydrocortisone on the quality of life in these patients.
Methods
About 883 patients with adrenal insufficiency were contacted, 526 patients participated (60%) and received a disease specific questionnaire and three standardized questionnaires (SF36, GBB24 and HADS). Reference data for SF-36 scores were obtained from the German National Health Survey comprising a representative random sample of 7124 subjects from the German population aged 18–79 years. Reference data for the GBB-24 ($n=2076$) and HADS ($n=2081$) were obtained from surveys performed by Brahler and colleagues. Finally, 282 patients on hydrocortisone therapy were grouped according their daily dose per body surface area (8–11 mg/BSA ($n=93$), 11–14 mg/BSA ($n=128$), 14–17 mg/BSA ($n=61$)) and analyzed regarding twice and thrice daily intake.
Results
In general, we observed a tendency towards better health related quality of life in patients taking hydrocortisone twice compared to thrice daily in all dose groups. The score of physical functioning as assessed by SF36 was significantly lower ($P<0.008$) and scores for exhaustion tendency, pain in the limbs and global score of discomfort assessed by GBB24 ($P<0.05$) were significantly higher in patients on a thrice daily intake compared to twice daily intake of 11–14 mg/BSA hydrocortisone indicating reduced health-related quality of life. However, anxiety and depression scores indicated no significant differences.
Conclusion
Health related quality of life tends to be impaired in patients on a thrice compared to a twice daily intake of hydrocortisone. It remains to be elucidated if a thrice daily intake is already a response of the physician to an impaired health status or if a thrice daily intake itself causes a reduced health related quality of life through mechanisms still unknown. Our results suggest a need for improved glucocorticoid replacement strategies.

P9

How does aldosterone renin ratio impact blood pressure levels? A Cross-Sectional Study of 3252 Normo- and hypertensive patients referred to coronary angiography

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Background
The renin-angiotensin-aldosterone-system (RAAS) is a major regulator of blood pressure, however, there are no studies available addressing its characterization in a large clinical setting. Therefore, the aim of the present study was to describe the relationship between parameters of the RAAS and actual blood pressure results in a large cohort of patients with and without essential hypertension.
Methods
We investigated 3253 patients (ages 63.2 ± 10 years) who were scheduled for coronary angiography in a single tertiary centre. We formed quartiles (QU) according to aldosterone/renin ratio (ARR; pg/ml).
Results
Sixty-nine percentage of the patients were hypertensive (s/dBP $\geq$140/90 mmHg) and mean systolic (sBP) and diastolic (dBP) blood pressure was 141 ± 23 and 80 ± 11 mmHg in the entire cohort. ARR in men was 10.1 ± 15.6 and in women 14.1 ± 19.7 ($P<0.005$). In a multivariate model, adjusting for age, sex, BMI, diabetes mellitus, NT-pro-BNP, daily activity, cystatin C, CRP, specific antihypertensive therapy mean sBP of ARR QU1 was 130.9 and increased to 147.2 mmHg in QU4. Diastolic BP increased significantly from 75.7 (QU1) to 85.1 mmHg (QU4), all P values <0.001. The overall influence of antihypertensive medication on ARR was rather small: ACE inhibitors decreased ARR to 9.8 (without ACE: 13.3), as well as diuretics (ARR 9.5 vs 12.2), whereas beta blockers increased ARR to 12.7 (vs 9.3) as did calcium channel blockers (13.9 vs 11.0). In a multivariate stepwise regression model overall predictable variance of sysBP was 28% (R2) and of dBP 22.6%. Here, the ARR was the single and second most important predictor of systolic and diastolic BP values.
Conclusions
ARR accounts for a large part of the variation in BP values and is also an important modulator of BP values in normotensive subjects.

P10

Effect of storage temperatures and different coagulants on stability of plasma ACTH

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The aim of the study was to investigate the effect of different coagulants and storage conditions on the stability of ACTH in plasma using the LIAISON-ACTH-Chemiluminescence Immunoassay.
Design and methods
Sequential human blood samples were collected from healthy volunteers ($n=83$) between 8 and 10 pm into EDTA collection tubes (Sarstedt) and EDTA plus aprotinin tubes (Vacuette, Greiner Labortechnik), respectively. After collection the blood samples were centrifuged immediately at 4 °C and on the other hand a second aliquot at room temperature, then assayed for ACTH immediately and after storage times of 2, 4, 8, and 24 h at 4 °C and other aliquots at room temperature.
ACTH was determined using the fully automated LIAISON random access analyser.
Results
EDTA-plasma samples collected with aprotinin centrifuged and stored at 4 °C showed the best stability of ACTH until 8 h with 94.0% in comparison to the ACTH concentration immediately after centrifugation; on the other hand centrifuged at 22 °C and stored at 22 °C the ACTH was stable for 4 h with 92.0%. ACTH in plasma samples without aprotinin centrifuged and stored at 4 °C was stable until 8 h with 92.6%; the ACTH centrifuged at 22 °C and stored at 4 °C was stable until 2 h with 99.0%; the ACTH was unstable after centrifugation and storage at 22 °C with a decrease at 2 h to 88.8%.
Conclusion
ACTH in EDTA-plasma after centrifugation at 22 °C is stable at 4 °C only for 2 h, the stability is extended until 8 h after centrifugation at 4 °C. The addition of aprotinin showed an improvement of the stability especially at 22 °C.

P11

Phosphodiesterase inhibitors and adrenal response to exercise-related stress

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Worldwide numerous active individuals take phosphodiesterase type 5 inhibitors (PDE-5i), furthermore, to our knowledge, the effects of these drugs on hormone secretion has not been adequately investigated. In particular,

considering that a) PDE-5i influence NO bioavailability, b) NO is one of the mediators of the hypothalamus–pituitary–adrenal (HPA) axis response to stress, and, c) physical exercise-related stress activate HPA axis, we investigated whether PDE-5i influence the salivary adrenal steroids responses to a maximal exercise test in healthy athletes. We studied 9 healthy male trained after signing the informed consent and our University Ethical approval. In the experimental phase, each subject performed, with a double blind design, two incremental exercise tests on a cycle ergometer, either after a single morning oral administration of one tablet of placebo or tadalafil (20 mg). Oxygen consumption (VO_2), blood lactate, respiratory exchange ratio, rate of perceived exertion, arterial blood pressure (BP), heart frequency (HR), and oxygen pulse (VO_2/HR), were evaluated immediately before and during exercise (at individual ventilatory and anaerobic thresholds, IVT and IAT), at VO_2 max and during recovery. Salivary cortisol, DHEAS, testosterone and their ratios (T/C, DHEAS/C) were evaluated by RIA before starting and at the end of exercise, and at thirty minutes during recovery. In contrast with placebo, tadalafil reduced systolic BP either before ($P<0.05$) and after exercise (at 3 min of recovery $P<0.05$) and decrease VO_2/HR at IVT ($P=0.03$). Salivary cortisol increased immediately after exercise both after placebo and after tadalafil administration ($P=0.04$ and $P=0.02$, respectively), with significant higher post-exercise salivary cortisol levels after tadalafil administration ($P=0.03$ versus placebo). Tadalafil administration was able to modify the salivary cortisol response to exercise related stress. Further studies are necessary to confirm our results. Administration was able to modify the salivary cortisol response to exercise related stress. Further studies are necessary to confirm our results. Tadalafil administration was able to modify the salivary cortisol response to exercise related stress. Further studies are necessary to confirm our results. Tadalafil administration was able to modify the salivary cortisol response to exercise related stress. Further studies are necessary to confirm our results.

P12

RNA interference inhibits VEGF and EG-VEGF expression of H295R human adrenocortical carcinoma cell line

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Background
The development of a vascular supply is a critical factor in the growth and metastatic spread of malignant tumors. The recognition of the vascular endothelial growth factor (VEGF) pathway as a key regulator of angiogenesis has led to a considerable efforts to exploit its potential for therapy in oncology. Endocrine gland-derived (EG-VEGF) has been recently identified as an endothelial cell mitogen, predominantly expressed in steroidogenic tissues; it represents a novel type of cell-specific effector that acts in a tissue-specific manner to promote angiogenesis. Both VEGF and EG-VEGF are over-expressed in adrenal carcinoma.
Objective
To inhibit the expression of VEGF and EG-VEGF in human adrenal carcinoma cell line H295R by RNA interference approach.
Methods
Designed oligonucleotides that contain the siRNA-expressing sequence targeting VEGF and EG-VEGF were annealed and inserted into the eukaryotic expression vectors pSuper and pSuper.neo (Oligoengine). After sequencing confirmation of the positive clones, the vectors were transfected into H295R cells and the levels of suppression of VEGF and EG-VEGF were measured by semi-quantitative RT-PCR and Real Time RT-PCR. Appropriate positive (GAPDH RNAi) and negative (scramble RNAi) controls were considered.
Results
In the H295R cells transfected with pSuper.neo-RNAi the amount of VEGF and EG-VEGF mRNAs was decreased by 55% and 40%, with respect to the cells transfected with the empty vector. Lower inhibition levels were found using the pSuper vector.
Conclusion
RNA interference inhibits the expression of VEGF and EG-VEGF in H295R providing a starting point for the biological therapy of adrenal cancer. As H295R cells express EG-VEGF receptors, we hypothesize that particularly EG-VEGF inhibition will result in decreased cellular proliferation. The effects of RNAi targeting VEGF and EG-VEGF on cells proliferation and apoptosis will be studied.

P13

The prevalence of non-classic adrenal hyperplasia among Tturkish women with hirsutism

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Context
The prevalence of NCAH among Turkish women with hirsutism has not been established so far.
Objective
To evaluate the prevalences of 21-hydroxylase (21-OHD) and 11-β hydroxylase deficiencies by ACTH stimulation test among hirsute women.
Patients and methods
The study population consisted of 285 premenopousal women, aged 16–46 years (mean: 23.2 ± 0.3). All were hirsute and hyperandrogenic. Androgen secreting tumors of the ovaries and the adrenal glands were excluded. All the patients were evaluated by 0.25 mg (i.v.) ACTH stimulation test and serum 17-hydroxyprogesterone (17-OHP) and 11-deoxycortisol (11-S) responses were obtained at 30 and 60 min. The diagnosis of NCAH due to 21-OHD was considered in patients in whom the post-stimulation 17-OHP level exceed 10 ng/ml and the cases found were confirmed by CYP21 genotyping. The diagnosis of 11-beta hydroxylase deficiency was made if the adrenal 11-S response to ACTH stimulation exceed three-fold the 95th percentile of controls. The 95th percentile for the 11-S response measured in our healthy subjects was 12.2 nmol/l.
Results
Eight (2.8%) and 18 (6.3%) of the patients had NCAH due to 21-OHD and 11-β hydroxylase deficiency, respectively. The rest of the patients were polycystic ovary syndrome (*n*: 160, 56.2%) and idiopathic hyperandrogenemia (*n*: 99, 34.7%).
Conclusion
This is the first and the most extensive national study investigating NCAH prevalence among Turkish population. It should be noted that the prevalence of 11-beta hydroxylase deficiency is higher than 21-OHD in women with hirsutism in this population.
This study was supported by TUBITAK (SBAG – 3170)

P14

Coincidence of pheochromocytoma and adrenocortical carcinoma at the same adrenal gland at patient with neurofibromatosis

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Introduction
We present the case of coincidence of pheochromocytoma and aderenocortical carcinoma at the same adrenal gland in patient with neurofibromatosis. That kind of tumors coincidence origin from different blastodermic layers is extremely rare.
It is broadly known that pheochromocytoma is rare neoplasm, occurs in 0.1% of hypertensive individuals. Pheochromocytoma may occur sporadically or in certain familial syndromes, including multiple endocrine neoplasia (MEN) 2A and 2B, neurofibromatosis, and von Hippel–Lindau (VHL) disease. Sporadic pheochromocytomas are usually unilateral, however in 10% of familiar tumors are located bilateral. The clinical manifestations of a pheochromocytoma result from excessive catecholamine secretion by the tumor.
Adrenocortical carcinoma is also very rare tumor which reveals in 1–2 cases per million. Etiology is still non-elucidated, but the role of genetic and environmental factors are mainly considerate. Most of the tumors are

functional and usually its first manifestation is Cushing syndrome with virylization.
Case report
The female 45-year-old patient with neurofibromatosis and bilateral tumors of adrenal glands was admitted to Endocrinology Department in order to confirm pheochromocytoma. The suspicion resulted from paroxysmal blood hypertension lasting for 1 year and arrhythmia. The hormonal examinations (metanephrine and vanillin-mandelic acid in the urine collection) confirmed pheochromocytoma. Remaining hormonal examinations were normal. After proper medical preparation the right adrenalectomy was performed. The histopatological examination revealed coincidence of pheochromocytoma and adrenocortical cancer at right adrenal gland. Controlled metanephrine urine collection again revealed increased level of normethanefrin and methanefrin. Two weeks after first operation the left adrenalectomy was preformed. Histopatological examination is in progress.
Conclusion
The coincidence of pheochromocytoma and adrenocortical carcinoma is unique. Finding of adrenocortical carcinoma cells in adrenal gland tissue during histopathological exam changes patients prognosis and planned follow up. Patients and patient's family members genetic exam should be considerated.

P15

The prevalence of 21-hydroxylase deficiency in adrenal incidentalomas: hormonal and mutation screening

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The aim of the present study was to evaluate and compare the response of 17 OHP to ACTH stimulation in patients with various types of adrenal incidentalomas and to examine the occurence of germline CYP21 mutation in these patients.
Subjects and methods
Forty patients (27 females, 13 males) with unilateral and bilateral masses were screened for five most common mutations of the CYP21 in peripheral blood DNA samples. A hormonal evaluation, i.e. baseline plasma values of 17OHP, DHEAS as well as plasma 17OHP and DHEA after ACTH stimulation, was performed in all patients.
Twenty-one of them had unilateral adrenal adenoma, 13 patients had adrenal hyperplasia (six of them unilateral) and 6 patients had CT characteristics of other tumors (myelolipomas, cysts, adrenocortical carcinoma).
Results
There were no significant differences in plasma 17OHP, DHEAS and plasma cortisol between all three groups. Stimulated plasma values of DHEA and 17OHP after ACTH administration were significantly higher in patients with adenomas ($P<0.05$ and $P<0.01$) and with hyperplasia ($P<0.05$ and $P<0.05$) compared with those with other tumors.
An exaggerated response of 17 OHP was found in 5 (12%) patients. However, mutation screening in peripheral blood samples revealed no CYP21 mutation in all examined groups.
Summary
Although 12% of patients with adrenal incidentalomas had an exaggerated response of 17 OHP after ACTH administration indicating a possible 21-hydroxylase deficiency, these findings are not associated with CYP21 mutation estimated in peripheral blood samples. There was found no germline CYP21 mutation in all patients with various adrenal incidentalomas.

P16

The role of midnight salivary cortisol levels in the diagnosis of subclinical hypercortisolism (SH) in patients with adrenal incidentaloma (AI)

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Background
Several criteria (i.e., cortisol supprimibility after 1 mg overnight dexamethasone suppression test -F-dex-, low ACTH levels, high 24 h urinary free cortisol levels - UFC-, high midnight serum cortisol levels) have been used for defining SH. Nevertheless, a real gold standard combination of tests is lacking. Recently midnight salivary cortisol (MSC) has been described as a sensitive marker for the diagnosis of overt hypercortisolism, while the role of MSC in the diagnosis of SH is not known. We evaluated the role of midnight salivary cortisol levels in diagnosing SH in AI subjects.
Subjects and methods
In 62 (F/M 38/24) patients with AI and in 45 (F/M 26/19) healthy controls matched for age, MSC levels were evaluated. In AI patients the evaluation of F-dex, UFC and ACTH plasma levels was also performed. We defined as affected with SH patients showing 2 out of the following: F-dex >83.0 nmol/l, ACTH <2.2 pmol/l, UFC >284 nmol/l. On the basis of these criteria, 12 patients showed SH (SH+) and 50 did not (SH−). The normal values of MSC are 0.7—5.7 nmol/l. The upper limit corresponds to the 95th percentile value of 45 controls. Data are expressed as mean$\pm$S.D.
Results
Age, BMI and percentage of males and females were comparable between SH+ and SH− patients. MSC levels were higher in patients with SH (4.6 ± 2.3 nmol/l) than in patients without SH (2.2 ± 3.1 nmol/l, $P=0.006$) and controls (1.7 ± 1.8 nmol/l, $P=0.001$). The MSC levels showed a significant correlation with F-dex ($r=0.28$, $P<0.05$). Using the cut-off of 5.7 nmol/l the sensitivity and the specificity of MSC levels for diagnosing SH is 33% and 90% respectively. In patients with AI normal levels of MSC do not exclude hypercortisolism whereas high levels can confirm the presence of SH suggested by conventional endocrine tests.

P17

Adrenal tumours in patients with a history of malignancy

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Background
Incidental discovery of an adrenal mass (AM) presents a common finding in patients with a history of extraadrenal malignant tumour. Discerning malignancy in AM is based on radiology (Rx) and scintigraphic (Sx) criteria, and finally fine-needle aspiration biopsy (FNA) of the tissue.
Objective
Determine the concordance of Rx and Sx techniques in patients with an AM and previous oncologic history.
Materials and methods
We carry out a retrospective study. We select 10 patients with AM discovered during the study of the primary tumour. To rule out functionality of AM, we perform an initial hormonal study. The lesions were discovered with abdominal computerized tomography and then we realized Sx study with ^{131}I cholesterol (except one case in which we used ^{75}Selenium). FNA was not realized in any case.
Results
Functionality of AM was ruled out in all cases. In 60% of cases existed concordance between Rx and Sx studies, and both techniques argued for benign nature (adenoma/hyperplasia). In 2 cases, the AM showed Rx signs (heterogeneous and >15 Hounsfield units) suggestive of malignant process. In opposition, the Sx showed bilateral uptake, indicative of benign disease. In one case, follow up of the patient during 7 years supported de benign nature of the AM, that remains stable in size and nonfunctional. In the second case the patient died because of causes related with the primary tumour. Death occurred after 2 years of follow up, and during this period, growth of the mass was not evidenced. In the last 2 cases, Rx characteristics were compatible with adenoma but the Sx features could not rule out metastasis because adrenal glands did not showed uptake. In one case the small size of the AM (1.5 cm) could explain the lack of uptake. In the last case, there was no explaining reason for no up take of the adrenal gland. However, the support of benign process by Rx and clinical signs and the difficult access by FNA, lead us to decide to wait and follow up. At the moment, stability of the AM indicates its benign nature.
Conclusions
In spite of progressive development of diagnostic tools, safety distinction between benign and metastasic ethiology of AM is in most cases extremely difficult. Establishing dignity in AM requires consensus and close collaboration of endocrinologists, radiologists, oncologists, and nuclear doctors to structure evaluation protocols to obtain maximum information from available diagnostic tools. Perhaps, near follow up of the patients will allow us to define nature of AM with enough certainty.

P18

Impact of total cumulative glucocorticoid dose on bone mineral density in patients with 21-hydroxylase deficiency

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Introduction
There are contradictory results concerning bone mineral density status in adult patients with congenital adrenal hyperplasia. To resolve this issue, we hypothesized that there could be a correlation between BMD and a total cumulative glucocorticoid dose from the diagnosis in early infancy to adulthood. We then conducted a retrospective in a referral centers for CAH. Thirty-eight adult patients (28 women, 10 men, aged 16–39 years) suffering from CAH and treated since early infancy (24 with the salt-wasting form, 5 with the simple virilizing form and 9 with the non-classical form) were included in the study. BMD was measured in the lumbar spine and femoral neck. Total cumulative dose (TCG) and total average (TAG) glucocorticoid dose were calculated from pediatric and adult files.
Results
CAH patients showed a significant difference between final height and target height (-0.82 ± 0.92 S.D. for women and -1.31 ± 0.84 S.D. for men; $P<0.001$). There was a discrepancy between the 28 women and the 10 men for lumbar T-score (-0.26 ± 0.23 S.D. versus -1.25 ± 0.42 S.D.; $P=0.04$) and femoral T-score (0.21 ± 0.25 S.D. versus -1.08 ± 0.35 S.D.; $P=0.009$). Non-classical women had higher lumbar T-score (0.33 ± 0.46 S.D. versus -0.36 ± 0.23 S.D.; $P=0.003$) and femoral T-score (0.62 ± 0.43 S.D. versus 0.20 ± 0.32 S.D.; $P=0.01$) than salt-wasting women. There was a strong correlation between BMI and femoral BMD in patients ($R=0.67$; $P<0.001$). We found negative correlations between TCG, TAG and lumbar ($R=-0.38$; $P=0.02$ and $R=-0.50$; $P=0.001$, respectively) and femoral T-scores ($R=-0.43$; $P=0.006$ and $R=-0.51$; $P<0.001$, respectively).
Conclusion
Adult CAH patients, especially men, have lower BMD and this was correlated with TCG. These results should help us in improving the management and the follow-up of BMD in CAH patients from childhood to adulthood.

P19

Angiogenic status of human adrenocortical tumors

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Introduction
Angiogenesis plays a major role in cancer growth and metastasis. Differences in angiogenesis and the balance of angiogenic growth factors and inhibitors may play a role in determining the observed variations in tumor behaviour. VEGF overexpression in adrenocortical carcinomas (AC) has been recently shown. Moreover a new steroidogenic specific tissue angiogenic factor (EG-VEGF) has been described, and its role is presently unknown in adrenal tumors. Previous evidence showed that somatostatin SST1 receptor (SSTR1) selective agonist inhibits VEGF and VEGFR2 expression.
Objective of the study
The aim of this study was to evaluate the expression of VEGF, VEGFR1, VEGFR2, EG-VEGF and SSTR1 genes in AC compared to aldosterone producing adenomas (APA) and normal adrenals (NA).
Methods
We analyzed the mRNA expression by real-time PCR in 11 AC, 20 APA and 7 NA.
Results

	VEGF	VEGFR1	VEGFR2	EG-VEGF	SSTR1
AC	°7/11; *3.6	°6/11; *3.5	°5/11; *4.5	°7/11; *52	°6/11; *19
APA	°11/20; *3.9	°12/20; *4.9	°8/20; *2.5	°9/20; *5.8	°17/20;*11

°positive /total samples; *fold-increase compared to controls (NA).

Conclusion
Our data show an over-expression of all studied genes (VEGF, VEGFR1, VEGFR2, EG-VEGF and SSTR1) in AC and APA compared to NA. The SSTR1 over-expression both in AC and in APA suggests potential therapeutic utility for SSTR1 selective agonist in the proliferative diseases involving angiogenesis. Finally we described, for the first time, a relevant EG-VEGF over-expression in AC compared to APA and NA. It will be interesting to evaluate the effect on EG-VEGF of the new inhibitors of tyrosin kinase which are currently used in anti-VEGF strategies in human tumors.

P20

Diagnostic potential of GC–MS urinary steroid profiling in the diagnosis of CAH due to 21-OH deficiency in neonates and infants

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21-hydroxylase deficiency is by far (>90%) the most common cause of congenital adrenal hyperplasia (CAH). Undiagnosed and untreated 21-hydoxylase deficiency bears the risk of salt loss, adrenal insufficiency and sex misassignment, therefore early diagnosis is very important. The most common way to identify children affected by the enzyme defect is the measurement of blood levels of adrenal hormones and precursor steroids, which is an invasive method and may lead to inaccuracy due to maternal-placental and fetal steroid products. We analyzed the urinary steroid profiles in neonates and young infants by GC–MS at time of diagnosis. The aim of the study was to determine the most specific and sensitive parameters of 21-hydroxylase deficiency. Twenty-seven children diagnosed with classical form of 21-hydroxylase deficiency,(14 boys, 13 girls; average age 37 (2–125) days) were included in the study and were compared with 47 healthy children (25 boys, 22 girls; average age 43 (0–144) days). Random urine samples were analyzed for steroid hormone metabolites and precursor/product ratios were calculated. Fetal zone steroids and cortisone metabolites were not discriminating. Indicators for 21-hydroxylase deficiency were: 15β,17α-dihydroxy-pregnanolone (15β,17α-OH-Po), pregnanetriol (PT), 11-*O*-pregnanetriol (11-*O*-PT), 5α,3α-pregnanolone (Po-5α,3α), 5β,3α-pregnanolone (Po-5β,3α). All parameters discriminated between CAH patients and healthy children by 100%. Differences were most expressed regarding 11-*O*-pregnanetriol.

Parameters	Conc. in CAH (μg/l) median (range)	Conc. in healthy (μg/l) median (range)	Cases detected
15β,17α-OH-Po	4177 (496–28 077)	22 (1–175)	27/27 (100%)
PT	1770 (183–17 715)	23 (3–133)	27/27 (100%)
11-*O*-PT	3390 (251–17 035)	4 (0–27)	27/27 (100%)
Po-5β,3α	1981 (186–17 677)	21 (0–63)	27/27 (100%)

Precursor/product ratios did not give more information to detect affected children. The advantage of urinary GC–MS analysis is that it is non-invasive and that it permits rapid and definitive diagnosis of 21-hydroxylase deficiency.

P21

11β-hydroxysteroid dehydrogenase 2 activity is elevated in extreme obese subjects and negatively associated with insulin sensitivity

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Objectives
Alterations in glucocorticoid (GC) metabolism may contribute to the development of obesity and insulin resistance. We aimed to study the role of

11β-hydroxysteroid dehydrogenase type 2 (11β-HSD2) in human adiposity, paying special attention to the association between altered GC metabolism and insulin sensitivity.
Design
In 24-h urine samples of 72 extremely obese (mean BMI 45.5±1.1 kg/m^2), but otherwise healthy patients urinary free cortisol (UFF) and cortisone (UFE), tetrahydrocortisol, 5α-tetrahydrocortisol, and tetrahydrocortisone were quantified by RIA. The sum of the three major tetrahydrometabolites is an estimate for daily GC secretion, and the sum of UFF and UFE represents potentially-bioactive-free-GCs. Thirty healthy lean subjects (BMI 22.3±0.3 kg/m^2) served as controls.
Results
In obese subjects, absolute daily GC secretion and the potentially-bioactive-free-GCs were significantly ($P<0.005$) higher than in lean controls (11.8±0.7 vs 8.0±0.6 mg/d; and 171.8±11.2 vs 117.6±9.2 μg/d, respectively). However, when these values were corrected for body surface area (BSA), significant differences were no longer detectable. While enzyme activity indices for 5α-reductase and 11β-HSD1 were similar in lean and obese subjects, 11β-HSD2 was markedly elevated in adiposity (3.7±0.2 vs 2.1±0.1; $P<0.0001$). This increase was accompanied by a significant reduction of UFF excretion corrected for BSA (16.5±1.2 vs 21.7±2.0 μg/d per m^2; $P=0.0222$). Besides, 11β-HSD2 activity was significantly correlated with insulin sensitivity ($P=0.0262$).
Conclusions
When body size is accounted for, both adrenal GC secretion and potentially-bioactive-free-GCs are indistinguishable between lean and extremely obese subjects. However, in obesity the kidney appears to intensify its supply of the direct substrate cortisone for extra-renal 11β-HSD1 which may fuel visceral adiposity and insulin resistance.

P22

Free fatty acids affect urinary excretion of androgen precursors thereby linking metabolism and hyperandrogenemia *in vivo*: results of a randomized, controlled trial

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Context
The polycystic ovarian syndrome (PCOS) is characterized by hyperandrogenism and associated with obesity and impaired glucose metabolism. Despite the high prevalence of PCOS and the considerable clinical impact, the precise interplay between metabolism and hyperandrogenemia is not entirely clear. We therefore aimed to analyse the relation between circulating free fatty acids (FFAs) and androgen metabolism *in vivo* in women.
Design and participants
Twelve healthy young women during the early follicular phase of two subsequent cycles were investigated. A randomized controlled cross-over trial was performed. Following a 10-h overnight fast, 20% lipid/heparin or saline/heparin infusion was administered at a rate of 1.5 ml/min for 5 h.
Main outcome measures
Circulating androgens and their precursors as well as 24 h-urinary excretion of free DHEA, DHEAS, androstenedione, androsterone (An), etiocholanolone (Et), 5-androstene-3β,17α-diol, 5-androstene-3β,17β-diol, DHEA, 16α-hydroxy-DHEA, and 5-androstene-3β,16α,17β-triol) were measured.
Results
FFAs induced elevated circulating levels of androstenedione, DHEA, DHEAS, testosterone, DHT, estrone and 17β-estradiol. Urinary excretion of DHEA, free DHEAS, 5-androstene-3β,17α-diol and sum of urinary excreted DHEA and its 16-hydroxylated downstream metabolites 16α-hydroxy-DHEA and 5-androstene-3β,16α,17β-triol were reduced by FFAs.
Conclusions
Elevation of FFAs increases adrenal androgen precursors DHEA and DHEAS by lowering their urinary excretion *in vivo* in healthy young women. The here described mechanism might contribute to the development of hyperandrogenism in women with PCOS and suggests novel therapeutic targets to treat those patients.

P23

Effects of free fatty acids on adrenocorticotropin secretion and metabolic glucocorticoid pattern in healthy young women

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Introduction
Free fatty acids (FFAs) affect pituitary function. While no effect of FFAs on ACTH and cortisol seems to exist in men, data in women are somewhat controversial. Therefore, we aimed to assess the effect of an acute increase of circulating FFA levels induced by the infusion of a lipid-heparin infusion on ACTH and cortisol secretion in healthy young women.
Methods
Following a 10-h overnight fast 13 healthy female volunteers were investigated in a randomised controlled cross-over trial. A 20% lipid/heparin (LHI) or saline/heparin (SHI) infusion was administered at a rate of 1.5 ml/min for 6 h. ACTH, cortisol and 24 h urinary excretion of glucocorticoids were measured to assess steroid metabolism, the overall daily cortisol secretion and the enzyme activities of 5α-reductase, 21-hydroxylase and 11β-hydroxysteroid dehydrogenase Type 1 and 2.
Results
During SHI, both serum cortisol and plasma ACTH showed a progressive decline according to the circadian rhythm. The cortisol levels were not altered by LHI, resulting in higher levels compared to SHI, while the decrease in plasma ACTH during LHI was comparable to the decline observed during SHI. No effects were found on daily urinary excretion rates of adrenal glucocorticoids, glucocorticoid precursors or calculated activity of 5α-reductase, 21-hydroxylase and 11β-HSD1 and 2.
Conclusion
FFAs seem to have no effect on ACTH secretion in normal weight young women. However the adrenal sensitivity to ACTH seems to be increased during LHI in women, as no change in neither in 21-hydroxylase activity nor in cortisol degradation, excretion or in peripheral conversion exists. This effect may have role in the development of obesity-associated complication.

P24

Association of diabetes mellitus and arterial hypertension in adrenal incidentaloma

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Background/objectives
The aim of the study was to assess association between diabetes mellitus (DM) and arterial hypertension (AH) in patients with adrenal incidentaloma.
Design and methods
One hundred and eleven consecutive patients with incidentally discovered adrenal tumours were included in the study. OGTT was done and 24 h urinary catecholamines were measured. Aldosterone and plasma renine activity (PRA) were determined, and 1 mg dexametasone test was performed. Insulin sensitivity (Si) was calculated using BIGTT test. Data are presented as mean±S.D.
Results
In our study group, there was significant association between DM and AH. Out of 13 DM subjects, 12 were also hypertensive. Out of 98 non-diabetic subjects 38 were normotensive and 60 hypertensive (Fisher exact test $P=0.031$). As there was only one normotensive diabetic patient two-way analysis was not feasible. Subjects with diabetes mellitus had significantly lower Si (7.32±3.05 vs 3.58±0.84×10^{-5} (min×pmol)$^{-1}$, $P=0.21$) and higher cortisol after 1 mg dexametasone (86.7±90.4 vs 155.0±192.5 nmol/l, $P=0.05$). Subjects with arterial hypertension were older (57.6±8.6 vs 52.7±9.9 years) and had significantly lower PRA (1.84±1.59 vs 3.85±3.52 ng/ml per h) compared to normotensive subjects.
Conclusions
In adrenal incidentaloma subjects significant association exists between DM and AH, probably caused by subclinical form of the Cushing's syndrome.

P25

Dehydroepiandrosterone (DHEA) exerts a prominent effect on chromaffin PC12 cell differentiation processes

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Within the adrenal gland, chromaffin cells and their progenitors are exposed to a wide variety of growth factors and hormones, including adrenal androgens such as DHEA. The DHEA producing-*zona reticularis* of the adrenal cortex is in close proximity to the neural crest-derived catecholamine-producing chromaffin cells of the medulla, enabling strong paracrine interactions. *In vivo* studies in humans revealed that congenital adrenal hyperplasia due to 21-hydroxylase (OH) deficiency results in androgen excess accompanied by severe adrenomedullary dysplasia and chromaffin cell dysfunction. Based on this evidence our *in vitro* study now aimed at further elucidating the effect of DHEA on chromaffin PC12 cells on a molecular level, focussing on cell survival and differentiation processes.
We could show that DHEA significantly reduced nerve growth factor (NGF)-induced cell survival as well as neuronal markers, such as neurite outgrowth and expression of neuronal marker proteins, like SNAP-25 and VAMP-2. Accordingly, DHEA was found to stimulate the NGF-stimulated cells towards a more neuroendocrine phenotype. Thus, DHEA largely elevated catecholamine release from NGF-induced PC12 cells and enhanced expression of the neuroendocrine marker chromogranin A. In a next step, we explored the molecular mechanisms of DHEA and NGF interaction in more detail. We could further demonstrate that DHEA significantly reduced NGF-mediated ERK1/2 MAPK activation. Differentiation as well as proliferation processes in PC12 cells are accompanied by ERK 1/2 activation.
In summary, our data demonstrate that DHEA influences differentiation processes in PC12 pheochromocytoma cells. DHEA drives the cells in the presence, but not in the absence, of NGF towards a more neuroendocrine phenotype. Our studies further suggest that this effect might be due to interference of DHEA with NGF-induced ERK1/2 activation by a rapid, so called non-genomic signalling mechanism.

P26

Glucocorticoid receptor gene polymorphisms and bone mineral density in hpa axis disorders

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Glucocorticoid receptor (GR) gene polymorphisms have been associated with interindividual variations in glucocorticoid sensitivity in healthy individuals.
Aim
Aim of this study was to investigate whether there was an association with sensitivity to glucocorticoids and bone mineral density in HPA axis disorders.
Methods
We analyzed 3 polymorphisms of the GR gene (Bcl1, N363S, ER22/23EK) in 185 subjects: 52 patients with Cushing's Syndrome (CS), 32 patients with adrenal incidentaloma (AI), 101 healthy subjects (C). The patients were compared by anthropometric and clinical indices and bone mineral density (BMD) at spine and femoral neck levels. Genotype frequencies were assessed for each polymorphism.
Results
The groups did not differ for age, sex, and BMI. Lumbar BMD was significantly lower in CS and AI compared to C, while there were no differences in femoral neck BMD. Lumbar and femoral neck z-score was significantly lower in CS compared to both AI and C. For N363S and ER22/23EK polymorphisms the allelic frequency was 1.89% and 0.81% respectively, so no associations with hormonal and bone parameters was evaluated. For BclI polymorphism, allelic frequencies were 28.1% in CS, 27.8% in AI, 41.2% in C. Heterozygous C/G was more prevalent ($P<0.03$) in CS and AI ($n=16/32$; $P<0.03$) than in C. After dexamethasone suppression test, CG carriers had lower cortisol levels than C/C carriers in CS, while no significant differences were found in AI and CG. Only in the lumbar spine a tendency towards lower BMD in CG carriers was found in AI, while no differences were found in femoral neck BMD and z-score. Lumbar and femoral neck BMD and z-score did not differ in C/G carriers with CS. In conclusion, we found an association between Bcl I polymorphism and GC sensitivity in subjects with Cushing's syndrome suggesting that this polymorphism might be associated with lower bone mineral density.

P27

Modulation of adrenocortical aldosterone and cortisol synthesis by *in vitro* oxidized low density lipoprotein

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Objectives
Oxidative stress is of critical importance in the pathogenesis of endocrinopathies. Since cholesterol serves as a major source of steroid hormone synthesis we investigated the effect of hypochlorite-modified low density lipoprotein (LDL) on aldosterone and cortisol release from human adrenocortical NCI-H295R cells.
Methods
Native LDL obtained from healthy volunteers was oxidized to varying degrees by sodium hypochlorite. The resulting modified LDL was biochemically characterized by gas chromatography–mass spectrometry (GC–MS) analysis. Human NCI-H295R cells were cultured in DMEM/F12. Aldosterone release in supernatants was measured by RIA and cortisol secretion was determined by competitive luminometric assay.
Results
Incubation of LDL with sodium hypochlorite resulted in increasing concentrations of the apolipoprotein B-100 oxidation markers HAVA, HACA, and 3-chlorotyrosine in dependence on the degree of oxidation. Incubation of adrenocortical cells with 10–100 μg/ml native or oxidized LDL for 24 h stimulated hormone release dose-dependently up to 3-fold. Subsequent stimulation of NCI-H295R cells with the physiological stimulus angiotensin II induced an additional hormone secretion up to 2.9-fold in LDL-pretreated samples. Compared to native LDL, oxidized LDL induced a smaller stimulation of hormone secretion that decreased with increasing degree of oxidation.
Conclusion
Oxidation of LDL may contribute to endocrine dysfunction by decreasing adrenocortical aldosterone and cortisol release.

P28

Establishment of a mutagenesis screen to identify mice with high aldosterone levels

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According to recent studies, primary aldosteronism is considered to be responsible for almost 10% of all cases of arterial hypertension. The genetic background of this common disease, however, has been elucidated only for the rare familial types whereas in the large majority of sporadic cases it still remains unclear. In an attempt to define novel genetic mechanisms of hyperaldosteronism we utilized a random mutagenesis screen after treatment with the alkylating agent *N*-ethylnitrosourea (ENU) and phenotypically characterized affected mice for their plasma aldosterone levels. As the detection method we used a time resolved fluorescence immunoassay, which allows the measurement of aldosterone in very small murine sample volumes. Using this assay we determined the normal aldosterone values for C3HeB/FeJ wild type mice under baseline conditions (mean ± S.D.; 92 ± 53 pg/ml for females ($n=69$) and 173 ± 114 pg/ml for males ($n=55$)) and following specific stimulation and suppression tests. Subsequently, aldosterone measurement was carried out in more than 2000 male and female F1 offspring of chemically mutated inbred C3HeB/FeJ mice. Upon repeated measurement, 7 female animals of the tested F1 offspring had consistently elevated aldosterone levels (defined as 3 S.D.'s above the mean of untreated

animals) measured at a mean of 456±104 pg/ml at initial time point. So far, no affected male mice have been detected. Further breeding of affected female animals gave rise to F2 pedigrees from which one established line displays high aldosterone values in 50% of littermates as would be expected from an autosomal dominant trait of inheritance. These animals will serve for detailed phenotypic and genetic characterization in the future. Taken together our data demonstrate the feasibility of a phenotype-driven mutagenesis screen to detect and establish mutant mouse lines with a high aldosterone phenotype.

P29

Late night salivary cortisol in the diagnosis of Cushing's syndrome

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Introduction
Late night salivary cortisol appears to be promising as a simple stress-free screening test for Cushing's syndrome (CS). But there is no consensus on the cut-off value because of the lack of availability larger studies and of standardization of assays. The normal reference ranges are assay-dependent and should be validated for each laboratory.
Objective
The purpose of this study was to evaluate the usefulness of late-night salivary cortisol in the diagnosis of CS.
Methods
We studied 49 normal subjects and 52 patients with suspected hypercortisolism. Salivary cortisol was collected at 0800 h and 2300 h using a commercially-available salivette and measured by an automated chemiluminescence assay. The study was approved by the Institute's ethical committee.
Results
Out of 52 patients 20 were confirmed to have CS and in the remaining CS was excluded by dexamethasone suppression test. The 2300-h salivary cortisol of patients with CS was significantly higher (1.24±0.86 μg/dl), as compared with patients in whom CS was excluded (0.109±0.076 μg/dl). The 0800-h cortisol was also significantly higher in CS (1.70±1.40 vs 0.240±0.132 μg/dl). The loss of circadian rhythm highlighted by 0800-h to 2300-h cortisol of < 2 was found in 17 of 20 CS patients. The upper limit of the reference range for 2300 h salivary cortisol was calculated nonparametrically as 0.311 μg/dl, from the data on normal subjects. Two of 20 patients with proven CS had 2300-h salivary cortisol less than the calculated upper limit of the reference range, yielding a sensitivity of 90%; one of these 2 patients had intermittent hypercortisolism.
Conclusion
Late-night salivary cortisol measurement is a simple and reliable screening test for Cushing's syndrome. A cut-off value of 0.311 μg/dl gives a sensitivity of 90% and specificity of 96.8%. Larger studies and wider availability of validated commercial assays are needed before it could be used as first line screening test.

P30

Hormonal, metabolic and bone evaluation in a series of adrenal incidentalomas

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Clinically silent adrenal masses discovered by abdominal imaging procedures performed for non-adrenal disorders, i.e. adrenal incidentalomas, have become a common finding in clinical practice and they represent a clinical concern because of the risk not only of malignancy but also of subclinical hormonal hypersecretion (SCS) that represents a new risk factor for cardiovascular diseases and/or osteoporosis. We studied 73 patients (39F, 34M; mean±S.E.M.: 61.6±1.3 year) with incidentalomas. In all patients the following measures were performed: urinary metanephrines, aldosterone/PRA ratio, UFC, cortisol, ACTH, DHEAS, 17OHP levels, Nugent and Liddle I tests. BMI, blood pressure, OGTT, serum lipids and DEXA were also measured. In 4 pts a biochemical diagnosis of pheocromocytoma was made. No case of primary hyperaldosteronism was demonstrated. In the others 69 pts, ACTH was inhibited in 8 pts, while UFC, cortisol, DHEAS and 17OHP levels were in the normal range in all pts. In 39 pts (57%) cortisol levels after Nugent test were < 1.8 μg/dl and no further hormonal evaluation was performed. In 7 pts (10%) post-Nugent cortisol levels were > 5.0 μg/dl and in 5 cases Liddle I test confirmed the existence of SCS. In 23 pts (33%) post-Nugent cortisol levels were between 1.8 and 5.0 μg/dl and Liddle I test in 13 cases confirmed the existence of SCS. The hormonal work-up demonstrated that 70% (51/73) of the masses were non-hypersecretory, 5% (4/73) were pheocromocytoma, 25% (18/73) were SCS. No significant difference was found in mass size, while a slight higher percentage of patients in the SCS group showed impairment in blood pressure, glucose tolerance, lipid profile and bone metabolism. Four SCS pts were operated, without a significant clinical improvement, while, in the other SCS pts, no significant progression of the disease was observed. Further studies on large populations are needed to clarify the clinical impact of SCS as well as the effects of adrenal mass removal.

P31

Follow-up in a group of 166 adrenal incidentaloma patients

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The adrenal incidentalomas have become a common clinical problem. In the vast majority of cases these masses are non- secreting adrenocrotical adenomas. However, some may show minor endocrine abnormalities with a subclinical hyperfunction or represent malignancies.
Objectives
The aim of the study was to analyze and follow-up of incidentalomas and to establish the risk factors for mass enlargement and/or hormonal disturbances.
Material and methods
One hundred and sixty-six patients, mean age 56, with adrenal incidentalomas were investigated in our Department in 1995–2006 years. Endocrine evaluation consisted of serum cortisol profile, ACTH, DHEA-S, aldosterone and plasma renin activity as well as 24 h urinary free cortisol, aldosterone and metanefrines excretion.
The adrenal tumors were revealed by ultrasound scan, MRI or CT scan of the abdomen performed for unrelated reasons. Body mass index and blood pressure were investigated. The follow-up period was in range of 1–10 years.
Results
Unilateral adrenal masses were found in 147 patients (right – 89, left – 58), while bilateral masses were present in 19 patients. Mean diameter of the adrenal mass was 3.6 cm (range 0.5–9). Ninety-four patients were overweight/obese (BMI > 25), 99 patients had hypertension.
In 133 patients the endocrine function was normal initially. Thirty-five persons demonstrated hormonal disturbances without clinical symptoms: 26 persons developed subclinical hypercortisolism, 5 showed increased 24 h urinary level of metanefrines and 2 exhibited hyperaldosteronism.
In 21 patients we observed enlargement of adrenal masses during follow-up.
The operation because of tumor size or hormonal function was performed in 83 patients. The adrenal cancer was diagnosed in 3 patients and bilateral metastases into adrenals in 3 persons.
Conclusions
A careful biochemical and imaging follow-up is advisable in all patients with adrenal incidentaloma because in some of them the hormonal hyperfunction or malignancy development is likely.

P32

Assessment of left ventricular functions by tissue Doppler echocardiography in patients with Cushing's disease

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Objective
To verify whether tissue Doppler imaging (TDI) could contribute to a better understanding of the natural history of cardiomyopathy in active Cushing's disease (CD), through its enhanced sensitivity to diastolic dysfunction,

and identifying preliminary regional signs of systolic dysfunction, before the appearance of clinical symptoms of cardiac pathologies.
Methods
Eleven women with newly diagnosed CD, and 15 control cases, purposely matched for gender, age, body mass index and co-incidental diseases were enrolled in this study. Conventional echocardiography and TDI examinations were performed with Vingmed System 7 (Vivid 7 Pro; Horten, Norway) using a 2.5-to 3.5-Mz transducer, and carried out by a single experienced cardiologist. The peak systolic velocity (S'm), early diastolic myocardial peak velocity (E'm), late diastolic myocardial peak velocity (A'm), isovolumic acceleration (IVA), myocardial pre-contraction time (PCT'm), myocardial contraction time (CT'm) and myocardial relaxation time (RT'm) were measured at septal (S) and lateral (L) mitral anulus.
Results
In TDI, E'mL, E'mS, E'mL/A'mL ratio, and E'mS/A'mS ratio were significantly lower ($P<0.05$), and PCT'mL/CT'mL ratio and PCT'mS/CT'mS ratio were higher ($P<0.05$), S'mL, S'mS, A'mL, A'mS, PCT'mL, PCT'mS, CT'mL, IVRT'mL and IVRT'mS values were similar in patients with CD than controls ($P>0.05$). Lateral and septal annulus IVA were significantly lower in patients with CD than the control group ($P<0.05$). Correlation analysis showed that IVA-L correlated positively with S'mL ($r=0.58$; $P=0.002$) and IVA-S correlated positively with S'mS ($r=0.51$; $P=0.008$).
Conclusion
The present study is the first study evaluating left ventricular functions in patients with active CD by TDI. We recommend using TDI, in addition to conventional echocardiography parameters for the cardiovascular risk assessment of patients with Cushing' syndrome.

P33

Salt wasting form of classic congenital adrenal hyperplasia (cah) due to 21-hydroxylase deficiency: an overview in adult life

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Background
Classic 21-hydroxylase deficiency, due to severe mutations in CYP21B gene, is an uncommon disease (1/ 150 000). Salt-wasting form accounts for 75% of the cases and turns up with gluco- and mineralocorticoid deficiency as well as feminine hermaphroditism. Although wide psycho-social repercussion especially in women is known, the rarity of the disease and losses in follow-up may lead to a scarce clinical experience. We present 3 adult patients with CAH- SW followed regularly at our outpatient clinic.
Case 1. 21-year male
Mutation in CYP21B: G656// Q318X/ R235W. Salt-wasting crisis in neonatal period. Frequent descompensations in childhood with banal infections. Phaco-emulsification of bilateral steroidal cataract at 16 years. Psycho-social: low academic performance, but he's working and leads an adequate social life. Height: 1.64 m, weight: 65 kg. Pectum excavatum. Low extremities' lymphedema. Current hormonal study: 17OHProgesterone: 20.8 ng/ml, plasma renin activity: 2.92 ng/ml per h, total testosterone: 6.75 ng/dl, free testosterone: 16.3 pg/ml.
Case 2. 18 year woman (sister of 1)
G656// Q318X/ R235W. Neonatal genital ambiguity. Clitoroplasty at 2 years old. Periodic vaginal dilatation. Frequent descompensations in childhood with banal infections. Menarche at 13 years, regular menses. Low academic performance, mutism, few and poor affective extrafamiliar relationships. 1.43 m, 66.5 kg. Low extremities lymphedema. 17OHP: 74.7, PRA: 3.27, androstendione: 2.9 ng/ml.
Case 3. 30 year woman
Delection CYP21B/del8pb. Neonatal genital ambiguity. Clitoroplasty and vaginoplasty at 2 and 4 years. Permanent urinary incontinence. Epilepsy. Multiple hospital admissions in child and adulthood. Osteoporosis. Menarche at 11 years, amenorreic periods. Personality disorder, uninhibited behaviour, hypersexuality, neglected hygiene, family dependence. 1.58 m, 75 kg. Severe hirsutism, red-wine striae, hyperpigmentation. 17OHProgesterone: 200, PRA: 20, androstendione: 8.
Comments
Management of adult patients with classic CAH needs to be tight, multi-disciplinary and specialized, having particular care for psychosocial adaptative disorders. Interaction of chronic steroid treatment and androgenic excess determines high morbidity mainly in affected women.

P34

Renal excretion rates of free cortisol, free cortisone and dehydroepiandrosterone metabolites, but not renal indices of cortisol secretion are associated with urinary volume in healthy children

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Background
In experimental studies, a high fluid intake and a corresponding high urine volume have been shown to increase renal excretion rates of urinary free cortisol (UFF) and cortisone (UFE) in adults. We aimed to examine whether 24-h urinary steroid excretion rates are also affected by urine volume in children.
Methods
In 24-h urine samples of 100 prepubertal and 100 pubertal healthy children UFF, UFE, tetrahydrocortisol, 5α-tetrahydrocortisol, and tetrahydrocortisone were quantified by RIA. Urinary dehydroepiandrosterone and its 16α-hydroxylated downstream metabolites (DHEA&M) were analyzed along with the above three glucocorticoid tetrahydrometabolites using GC–MS in two additional groups of prepubertal ($n=100$) and pubertal ($n=100$) children. The sum of the 3 primarily glucuronidated tetrahydrometabolites (GC3) reflects daily cortisol secretion and DHEA&M represents an index of adrenarchal androgen secretion. Associations of urine volume with outcome variables UFF, UFE, GC3, and DHEA&M were examined in each developmental group using multiple regression models adjusted for sex, body weight, height, and total energy intake.
Results
Significant positive associations were observed between 24-h urine volume and UFF, UFE and DHEA&M in the respective prepupertal and pubertal groups with the highest explained variation for UFE, especially in puberty ($R^2=0.24$). However, GC3 was not significant in any of the groups.
Conclusion
Urinary 24-h excretion rates of UFF, UFE, and DHEA&M, but not glucocorticoid secretion parameters are affected by daily urine volume in healthy free-living children. For a more specific assessment of associations of UFF, UFE, or DHEA&M with (patho)physiologically relevant factors, urine volume should be considered as a confounder.
This work was supported by a grant from European Chemical Industry Council (Cefic).

P35

Glucocorticoids and body fat are associated with urinary excretion rates of uric acid and oxalate, but not of calcium in healthy children

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Background
In patients with hypercortisolism, who are frequently obese, the prevalence of urolithiasis is increased and urinary excretion rates of calcium (Ca), oxalate (Ox), and uric acid (UA) are regularly elevated. In the present study, we examined whether these lithogenic factors are already associated with dietary intakes, body fat, and cortisol metabolites in free-living children.
Methods
In 24-h urine samples of healthy children (150 boys, 150 girls, aged 4–14 years) urinary free cortisol (UFF), cortisone (UFE), the sum of 3 major glucocorticoid metabolites (GC3) as well as Ca, Ox, UA and net acid excretion (NAE) were determined cross-sectionally along with relevant nutritional and anthropometric parameters. Potentially-bioactive-free-glucocorticoids were assessed as UFF+UFE and adrenal glucocorticoid secretion as GC3. Associations of diet, percent body fat (%BF) and glucocorticoids with outcome variables were examined in multiple regression models adjusted for sex, age, height, growth velocity and total energy intake.
Results
Positive associations with the urinary outcomes Ca, Ox, and UA were observed for the nutritional factors Ca, fiber, and protein intake, respectively, for sodium intake (outcomes: Ca, UA), and for the indicator of dietary acid load NAE (outcomes: Ca, Ox, UA). %BF and cortisol secretion (GC3) were both positive predictors of UA and Ox. Of all dietary and hormonal variables, UFF+UFE explained most of the variation (partial

$R^2=0.08$, $P<0.0001$) of urinary UA and also part of the variation of Ox (partial $R^2=0.02$, $P<0.05$), but none of Ca.
Conclusion
Apart from the known nutritional determinants, such as protein and sodium intake, also dietary acid load and increased body fatness appear to affect urinary Ca, Ox and UA. Additionally, higher potentially-bioactive-free-glucocorticoids, even in the physiological range, may contribute to stone forming risk via elevations in UA and Ox, but not Ca.

P36

Aberrant cortisol responses in adrenal incidentalomas: a study on evaluation and management of 20 patients

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In overt or subclinical ACTH-independant Cushing's syndrome (CS), aberrant adrenal receptors may control cortisol secretion.
We systematically studied the 20 patients with adrenal incidentaloma screened in our department for illegitimate adrenal receptors between 2000 and 2006. We investigated plasma cortisol level during successive stimulation tests performed while dexamethasone was given orally every 8 h (upright posture, meal, hypothalamic hormones, terlipressin, angiotensin II, metoclopramide and/or octreotide). Subjects were considered as responders if their plasma cortisol level increased at least by 25% after one or several tests without any parallel elevation of ACTH.
Fourteen patients were responders, 11 of them responded to several tests. The most constant responses occurred after terlipressin (85% of explored subjects), suggesting aberrant vasopressin receptors in these patients. Neither clinical, biological or hormonal features nor adrenal tumour dimension nor noriodocholesterol uptake were predictive of illegitimate cortisol response. The responders rate did not differ significantly between unilateral adrenal adenomas (69.2%, $n=13$) and bilateral macronodular adrenal hyperplasias (71.4%, $n=7$). Ten patients with features of subclinical CS, including eight responders, underwent adrenal surgery. Metabolism abnormalities and bone mineral density did not improve clearly afterwards.
In 3 prospective studies evaluating aberrant adrenal receptors in subclinical CS, each patient presented with illicit cortisol response(s). In accordance with our results, vasopressin and its analogues induced the most constant responses in these studies. They differed from our procedure in 2 points: dexamethasone was administered during explorations in only two of these studies and cortisol responses mediated through an elevation of plasma ACTH were excluded in none of them. Adrenalectomy is still controversial in the management of subclinical CS. In our study, it resulted in no obvious metabolic or bone benefit. In the future, medications targeting aberrant responses may be an alternative for these patients.

P37

The German Conn's registry: comorbidities in over 700 patients with primary hyperaldosteronism

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Objective
Hypokalemic Conn's syndrome is a rare disease with a prevalence of 0.5% in unselected hypertensive populations. However, recent studies indicate a higher prevalence of a milder variant of Conn's syndrome, reaching 10% in some studies. Long term outcome and health care costs of hypo- and normokalemic variants are largely unknown. The National Conn's Registry is an initiative to create a national database of sufficient epidemiological strength to investigate co-morbidity and mortality in these patients.
Methods
The registry has at present 7 participating centres in 5 locations and uses an electronic database to assure comparison of different centres. Since 07/2006 retrospective data were included in the database from patients with Conn's syndrome diagnosed between 1990 and 2007.
Results
Evaluation of the retrospective data entry provide the following results: Of the 726 patients (60.4 ± 13.7 years, range 6–96 years), 54.3% had the hypokalemic variant of the disease. The mean RR was 158 ± 29 mmHg systolic and 94 ± 16 mmHg diastolic. Morbidity of cerebrovascular events (TIA, PRIND, stroke) in the overall cohort was high with 9.6% of patients. The prevalence of cardiovascular morbidity (angina pectoris, myocardial infarction, coronary angioplasty) was 13.5% in our cohort. Atrial fibrillation occurred in 8.4% of the patients and other atrial or ventricular dysrhythmia in 5.5% of the patients. Chronic renal failure was present in 10.9% of patients, and sleep apnea in 8.1% of patients. Overall co-morbidities were more frequent in hypokalemic than in normokalemic individuals.
Conclusion
Our data show high proportion co-morbidites for Conn's syndrome, which is endorsed by previous results from France describing a 3–4 fold higher prevalence of stroke, myocardial infarction and atrial fibrillation in a small cohort of primary aldosteronism ($n=124$) compared to patients with essential hypertension ($n=465$). In addition, our data demonstrate evidence that the hypokalemic variant of Conn's syndrome has a higher morbidity than the normokalemic variant.

P38

Retrospective clinical data on more than 160 pheochromocytoma in three east german endocrine centers

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Objective
Pheochromocytoma is a rare disease with an incidence of 2–6 per million, and a prevalence of 0.1–0.6% in patients with hypertension. Pheochromocytoma may occur sporadically or as part of hereditary syndrome. According to the latest studies, among patients with non-syndromic pheochromocytoma, up to 24% of tumors may be inherited. However, genetic testing was not generally performed until recently. There is still a debate which pheochromocytoma patient should be tested for hereditary syndrome. We started an initiative to create a database of sufficient epidemiological strength to investigate pheochromocytoma in more detail.
Methods
The preliminary registry has at present 3 participating centres in 3 locations and uses an electronic database to assure comparison of different centres. Since 07/2006 retrospective data was included in the database from patients with pheochromocytoma between 1973 and 2007.
Results
Evaluation of the retrospective data entry shows the following results: Of the 166 patients (49.3 ± 14.4 years, range 12–81 years, 49.4% males), 115 pheochromocytomas were unilateral (64 right, 53 left), 19 bilateral, and 4 extraadrenal (no information in 28 patients). The mean size of unilateral pheochromocytoma was 64 ± 25 mm. Fourteen pheochromocytoma were diagnosed as malignant, with only 10 patients showing evidence of metastasis. Genetic testing was performed in only 31 patients revealing a hereditary syndrome in 13 patients (8 RET, 3 VHL, 2 NF1). In addition, a hereditary syndrome was diagnosed clinically in 21 patients (14 MEN, 4 VHL, 3 NF1).
Conclusion
In general, documentation and follow-up of patients with pheochromocytoma was insufficient over the recent years. This emphasizes the need for a clinical national registry including follow-up information of the patients. In our cohort 34 patients (20.5%) had a hereditary form of pheocromocytoma, which is in accordance to previous studies.

P39

Impaired endothelial morphology and function in patients with subclinical Cushing's syndrome due to single adrenocortical adenomas

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Background
Subclinical Cushing's syndrome (SCS) due to incidentally discovered adrenal adenomas (AI) has been associated with increased prevalence of hypertension, obesity, and impaired glucose tolerance which are established risk factors for cardiovascular morbidity. Although functional and morphological (structural) changes of endothelium have been correlated with cardiovascular morbidity this has not been looked into detail in patients with adrenal incidentalomas.
Subjects and methods
Endothelial morphology and function was studied using high resolution linear array ultrasound by measurement of carotid artery intima-media-thickness (IMT) and brachial artery flow-mediated dilation (FMD) in 15 patients with SCS due to AI (54.8 $\pm$ 2.3 years, BMI 27.9 $\pm$ 1.07 kg/m^2) (mean $\pm$ S.E.M). Twenty age, gender and BMI matched patients with non functioning AI (NFAI) (53.1 $\pm$ 2.2 years, BMI 27.3 $\pm$ 0.6) were also studied and served as controls. Patients with SCS had incomplete inhibition of serum cortisol levels after a formal low dose 2 days 2-mg DXM suppression test. All subjects had no evidence of cardiovascular disease and/or diabetes mellitus and had normal 24 h urine catecholamine levels and no evidence of autonous aldosterone secretion. Systolic and diastolic blood pressure and the following fasting concentrations were measured: glucose, insulin, triglycerides, total cholesterol, high and low density lipoproteins, fibrinogen, homocysteine. Insulin sensitivity was assessed by the homeostasis model assessment index (HOMA).
Results
Patients with SCS had higher IMT values than patients with NFAI (0.99 $\pm$ 0.07 mm vs 0.83 $\pm$ 0.03, $P = 0.038$) and lower FMD levels (3.1 $\pm$ 0.44% vs 4.3 $\pm$ 0.38, $P = 0.039$) suggesting both functional and morphological endothelial dysfunction. HOMA index was significantly lower in SCS (1.94 $\pm$ 0.34 vs 3.05 $\pm$ 0.33 mmol/mU per l, $P = 0.039$) reflecting a significant reduction in insulin sensitivity. No differences were found in fasting glucose levels, lipid profile and blood pressure.
Conclusion
Patients with mild subclinical autonomous cortisol excess due to AI exhibit impaired endothelial function and morphology. This could be linked to subsequently increased risk for cardiovascular diseases.

P40

Comparison of strategies for biochemical diagnosis of primary aldosteronism in German academic centres

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Recent studies indicating that normokaliemic primary aldosteronism (PA) is a more frequent cause of hypertension than previously expected led to an increased interest in biochemical screening strategies. We investigated biochemical diagnostic strategies used in different academic centres for patients documented in the German National Conn's Registry. Data from 7 centres in 5 cities have been entered into a database by trained personnel. For analysis, results from 522 patients with documented results of measurements of aldosterone (A) in plasma or urine, renin concentration (RC) or renin acitivity (RA) were used, 85% of the results being obtained between 1997 and 2006. Aldosterone to renin ratio (ARR) was documented in 81.4% of patients, but used as first diagnostic step in only 64.7% (range 43.3–79.1% depending on centre). In 35.3% (range 20.9–56.7%) of patients, the first documented biochemical result was obtained already during a confirmatory or differential diagnostic test (salt load, posture test, lasix-renin test, adrenal vein sampling). Urinary aldosterone was analysed in only 4.6% of patients. ARR was documented in 66.7% (range 89.4–43.6%) during the first week of diagnosis. Documentation of variables potentially influencing ARR was poor (medication 70%, *K* 42.5%). Medication was adequately paused in 93.2% (mineralocorticoid receptor antagonists) and 59.4% (beta blocker). About 67% of patients were normokaliaemic at ARR determination. ARR values were calculated from 8 different combinations of assays (A plus RA or RC), with one or more changes in methods during the observational period in 6 of 7 centres. Mean A, RC and RA concentrations differed significantly between methods, as did the corresponding ARR. The highest ARR was observed for the combination Adalits A/Adaltis RA and DPC A/Nichols RC. In conclusion, diagnostic strategies differed between centres, which might also be reflected in a different composition of patient's populations.

P41

Addison's disease: aetiological role of oncological factors

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Autoimmune destruction of the adrenocortical cells is the most frequent form of Addi-son's disease, however modern imaging methods make possible to discover bilateral neoplastic laesions resulting in adrenal insufficiency more frequently than about twenty years ago. In our group of 315 patients with Addison's disease we diagnosed metastatic infiltrations of the adrenals in 18 cases and lymphoma of both adrenals in four cases (in sum 7%). Typical clinical signs, biochemical abnormalities and results of hormonal examinations (low cortisol, high ACTH levels, lack of adrenal reserve during ACTH stimulation) were present in majority of them. In six cases pre-Addison's disease was diagnosed, presenting with fatigability and increased ACTH concentrations.
A specific form of adrenal insufficiency has been observed in patients with adrenal cancer, treated with mitotane, an adrenolytic agent. In such cases higher replacement doses of hydrocortisone, than in classic Addison's disease, were necessary (quick conversion to inactive forms of steroids, increased transcortin levels). In our material of 194 patients with adrenal cancer mitotane was administered in a long-term therapy in 168 cases. In 18 out of 50 survivors it was possible to withdraw mitotane within four to five years of therapy, however in two patients a continuous adrenal insufficiency has been observed.
Conclusions
A relationship of adrenal insufficiency with oncology is evident however it is not a frequent finding.

P42

Diurnal salivary cortisol in young adult-onset diabetes mellitus Type 1 patients

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Detailed information on adrenal function in autoimmune Type 1 diabetes with onset in adult age is still missing. This work compared diabetics with low response (LR) to diabetics with normal response (NR) to low dose ACTH test and control group (C).
Thirty-two diabetics were investigated; LR ($n = 16$), NR ($n = 16$), age 44 $\pm$ 10 years (mean $\pm$ S.D.), age at diagnosis 28.5 $\pm$ 10 years, disease duration 15 $\pm$ 8 years, BMI 24.5 $\pm$ 2.7 kg/cm^2, HbA1c 7.2 $\pm$ 1.2%. Control group consisted of 16 healthy subjects; age 27 $\pm$ 6 years, BMI 21.7 $\pm$ 2.3 kg/cm^2. Neither group showed any clinical signs of adrenal disorders or adrenal laboratory autoimmunity. The study was approved by local Ethical Committee. Adrenal reserve was tested by low dose ACTH test. Adrenal autoantibodies, plasma renin activity, transcortin (CBG) were determined. Diurnal rhythm of salivary cortisol was investigated at 8 am, 12 am, 5 pm and 10 pm.

Basal and stimulated serum and salivary cortisol levels in NR did not differ significantly from those in C. Diurnal rhythm of salivary cortisol in NR did not differ from C. In LR, basal and stimulated levels of serum and salivary cortisol were significantly lower than C and NR, $P<0.001$ for all times. Diurnal rhythm was changed, the levels of salivary cortisol were lower than NR and C, at 8 am and at 12 am $P<0.05$. LR did not differ from NR and C in either basal ACTH value, or basal plasma renin activity, but levels of CBG was significantly lower than NR, $P<0.005$.

In conclusion, low response to low dose ACHT test was associated with changes in diurnal rhythm of salivary cortisol and lower levels of CBG. These changes support the conclusion that a portion of diabetics even without adrenal autoimmunity show adrenal function impairment. The clinical significance remains to be evaluated. The study was supported by grant No.NR/9154-3 IGAMZCR.

P43

Insulin sensitivity calucated by HOMA, QUIQI and G/IR in patients with adrenal incidentaloma

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Many studies have shown insulin resistance (IR) in majority of patients with adrenal incidentaloma. The euglycaemic hyperinsulinaemic glucose clamp technique is still considered as 'golden standard' for evaluation of insuline sensitivity. These methods are also complicated, labor-intensive procedure better for small research studies. Today, several surrogate indexes for insulin sensitivity were introduced such as: HOMA, QUIQI or G/IR. Some studies showed that QUIQI had significantly better linear correlation with reference glucose clamp method.

The aim of our study was to analyze insulin sensitivity and compared results and prevalence of insuline resistance by various methods.

About 208 patients (148 women and 60 men, mean age 55.08 ± 11.02 years and mean BMI: 27.91 ± 4.6 kg/m^2) with adrenal incidentaloma were admitted to hospital. Insulin sensitivity was calculated by HOMA, QUIQI and G/IR method. Mean insulin sensitivity: HOMA 3.61 ± 6.61; QUIQI 0.34 ± 0.04 and G/IR 10.59 ± 17.15. According to criteria insulin <12 mU/ml, glucose/insulin >6.4, HOMA <4.7, and QUICKI >0.333, insulin resistance was present in 56.84% of patients calculated by HOMA and QUIQI and 43.16% of patients calculated by G/IR method.

No statistically difference was found comparing these methods in insulin sensitivity (Mann Whitney Test: $W=830$; $P=0.626$), and in prevalence of IR (Person X^2 test$=0.772$, df$=1$; $P=0.782$) among patients with adrenal incidentaloma.

Our study shows significant prevalence of IR in patients with adrenal incidentaloma. Prevalence of IR with G/IR was smaller than with HOMA and QUIQI methods but not statistically significant.

P44

Quantitative real time RT-PCR of CYP11B2 (aldosterone synthase) to confirm the diagnosis of aldosterone-producing adenomas

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Background

The diagnosis of hyperaldosteronism is hindered by the absence of a well-defined gold-standard. CYP11B2 (aldosterone synthase) belongs to the steroid-metabolizing enzymes catalyzing the last step of the aldosterone synthesis. To establish a molecular marker for aldosterone-producing adenomas, we compared the expression in various adrenal tumors.

Methods

Tissue from 20 aldosterone-producing adenomas (APA), 12 hyperplasias associated with hyperaldosteronism (H), 20 cortisol-producing adenomas (CPA), 20 pheochromocytomas (PHEO), and 20 non-functional adenomas (NFA) was obtained following laparoscopic surgery. The diagnosis was confirmed by various biochemical tests, histological investigation, and clinical follow-up. Extracted RNA underwent Real Time RT-PCR using CYP11B2 specific primers and probe (detection limit 5×10^1 copies/μg RNA (cp)). mRNA levels were normalized to GAPDH mRNA levels. ROC analysis was performed to established cut-offs with specificity of at least 95%.

Results

APA demonstrated high CYP11B2 expression with a median of 1.4×10^8 cp (range 6.1×10^5–1.9×10^{11} cp). In contrast, expression was significantly lower ($P<0.001$) in CPA, PHEO, and NFA with 5×10^1 cp (5×10^1–8.9×10^6 cp), 4.7×10^2 cp (5×10^1–2.3×10^7 cp), and 5×10^1 cp (5×10^1–2.5×10^6 cp), respectively. ROC analysis suggested a threshold of 1×10^7 cp with a sensitivity of 90% and specificity of 97%. In H, CYP11B2 expression levels ranged from 5×10^1 to 2.7×10^9 with a median of 1.9×10^5 cp. Inclusion of H to the ROC analysis led to an identical cut-off with lower sensitivity (69%) and similar specificity (97%).

Conclusion

Characterization of CYP11B2 expression may serve as a molecular marker to distinguish aldosterone-producing adenomas and bilateral hyperplasia from other adrenal neoplasms. Such criteria could be used to evaluate biochemical tests for the diagnosis of hyperaldosteronism. Diffuse hyperplasia of both adrenals in H may lead to hyperaldosteronism even in the presence of low expression of CYP11B.

P45

Renal function in patients with primary aldosteronism: comparison with essential hypertension

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Objective

Primary aldosteronism (PA) is associated with vascular end organ damage. We evaluated the newly established German Conn's Register for evidence of renal impairment and compared the data with renal function from hypertensive subjects from an epidemiologic cohort.

Methods

The registry was founded in 2006 and has at present 7 participating centres in 5 locations. Data are entered in a central electronic database. Up to July 2007 555 patients were enrolled and matched for age, sex and BMI in a 1:1 ratio with hypertensive control subjects form the epidemiologic F3 survey of the Kooperative Gesundheitsforschung in the region of Augsburg (KORA). About 408 patients with PA and 408 hypertensive controls were finally analyzed.

Results

The percentage of patients with a serum creatinine concentration above the normal range of 1.2 mg/dl was significantly higher in patients with PA than in hypertensive controls (129 of 408 (31.6%) vs 48 of 408 (11.8%), $P<0.001$). Accordingly, mean glomerular filtration rate (GFR) was significantly lower ($P<0.001$). Subgroup analysis showed that this was independent of sex, age or diabetes, but hypokalemic patients with PA had lower GFR than normokalemic patients. In a second step we analysed the long-term effect of specific treatment on renal function. Adrenalectomy for aldosterone producing adenoma reduced systolic blood pressure from a mean of 160 to 143.7 mmHg. In parallel, we observed an increase in serum creatinine and a decrease of GFR from 71.4 to 64 ml/min ($P<0.001$). A similar trend was seen with spironolactone treatment.

Conclusions

Glomerular filtration rate is reduced in patients with PA. Normalizing systemic blood pressure by removal of the adenoma reduces renal plasma flow. This uncovers the real extend of renal impairment in PA.

P46

Adrenal retinoid receptors: new findings and new questions

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Retinoid-X-receptor (RXR) and retinoic acid receptor (RAR) are transcription factors; each expresses in α, β and γ isoforms. Whereas RAR plays roles in growth and differentiation, RXR regulates lipids homeostasis. Specifically, RXRβ mediates intracellular control of cholesterol levels. RXR is activated by 9-*cis*-retinoic acid (9-*cis*-RA), cervonic acid, docosapentaenoic acid, adrenic acid and arachidonic acid. The present investigation was aimed to characterize adrenal retinoid receptors. Adrenals were collected from foetus (20th day of pregnancy), neonate and adult rats, and from the adult rats stimulated with ACTH (30 μg/100 g) for four consecutive days. Retinoid receptors were studied by western blotting. Cholesterol ester (CE) was determined by HPLC. Fatty acids and retinoic acids were analyzed by GC/MS and LC/MS, respectively. The results showed that development of rats from foetus/neonate to adult was accompanied by increased accumulation of CE and expression of RXRα, RXRβ and RARγ in the gland. In contrast, adrenal expression of RARα diminished. Ligand analysis revealed all-*trans*-retinoic acid, cervonic acid, docosapentaenoic acid, adrenic acid, and arachidonic acid; 9-*cis*-RA was not detectable. Based on these ligand-availabilities, RAR/RXR and RXR/RXR dimerizations could both exist in the gland; their specific isoforms in partnerships remained unknown. ACTH-stimulation resulted in altered adrenal retinoid receptor levels, but without a common pattern. Adrenal RXRβ was concentrated in the nuclear fraction, but was also found in the pellet resulted from a high speed centrifugation of the post-nuclear fraction, which could not entirely be explained as 'contaminated'. No ACTH-induced translocation of RXRβ between nuclear and cytosolic fractions was observed. Despite new questions raised, the present data suggest a possible involvement of the retinoid receptors in regulation of rat adrenal development and steroidogenesis.

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Screening for aberrant peptide hormone responsiveness in primary aldosteronism

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Recent molecular studies suggest a role of ectopic G protein coupled receptors in primary aldosteronism (PA). The clinical relevance of these findings is not known. We therefore combined expression profiling and a clinical testing of hormone responsiveness in patients with primary aldosteronism. Sixteen tissues from aldosterone producing adenomas and 6 normal adrenal glands were subjected to a quantitative PCR for determination of mRNA expression levels of AT2R, GIPR, MC2R, GnRHR, LHR, TRHR, TSHR, Glucagon-R (GCGR), AVPR and 5HT4R. Twelve patients with confirmed primary aldosteronism due to adrenal adenoma (APA; $n=5$) and bilateral adrenal hyperplasia (BAH; $n=7$) and 8 control subjects (C) could be enclosed in a test protocol consisting of 8 stimulation tests on three consecutive days, including stimulation by posture, mixed meal, ACTH, GnRH, TRH, glucagon, vasopressin and metoclopramide (MCP), respectively. Most APA tissues displayed ATIIR (16/16), MC2R (15/16), 5HT4R (15/16) and. AVPR (13/16) mRNA expression. In contrast, only in a minority of tissues GnRHR (4/16), LHR (1/16), TSHR (1/16), GIPR (0/16), GCGR (0/16), or TRHR (0/16) mRNA was detectable. Clinical testing revealed responsiveness of aldosterone secretion following ACTH (APA, 5/5; BAH 7/7), MCP (APA, 4/5; BAH 7/7), posture test (APA, 1/5; BAH 3/7), mixed meal (APA, 0/5; BAH 0/7), TRH (APA, 0/5; BAH 1/7), vasopressin (APA, 2/5; BAH 4/7), glucagon (APA, 1/5; BAH 1/7) and GnRH (APA, 1/5; BAH 2/7). In three patients where both data were available, clinical and molecular studies were well correlated, especially in the APA patient with GnRH responsiveness where GnRH expression was detectable. We conclude that peptide hormone responsiveness is a common finding in patients with primary aldosteronism based on clinical testing and peptide hormone receptors expression profiling. Whether hormone receptor expression is sufficient to induce a clinically relevant autonomous aldosterone secretion remains to be determined.

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Persistent increase of osteoprotegerin levels after cortisol normalization in patients with Cushing's syndrome

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Osteoprotegerin (OPG) has been identified as a decoy receptor that inhibits osteoclast differentiation and recently as a paracrine regulator of vascular calcification. OPG is suppressed by glucocorticoid administration. In this study, we evaluated OPG and bone metabolism in Cushing's syndrome (CS) before and after surgical treatment. Twenty-nine patients with CS (26 women and 3 men, mean age: 40.7–2.8 years) and 27 age and sex-matched controls have been studied for bone mineral density, bone metabolism, OPG and receptor activator of nuclear factor-kB ligand (RANKL). Sixteen patients were studied for atleast 18 months after surgery, with normalization or reduction of cortisol levels. BMD was significantly lower in CS than in controls (lumbar spine:0.6 ± 0.02 and 1.01 ± 0.02, $P<0.0001$; femoral neck: 0.73 ± 0.22 and 0.81 ± 0.2, $P=0.02$). OPG levels were significantly increased in CS than in controls (4.5 ± 0.4 and 3.2 ± 0.3 pmol/l; $P=0.02$). Ca, *P*, osteocalcin (OC) and PTH did not differ between CS and controls. A significant positive correlation was found between serum cross laps and OC levels ($r^2=0.43$, $P<0.01$) and between total alkaline phosphatase and RANKL ($r^2=0.42$, $P<0.005$). After treatment we found no difference of OPG levels and a significant increase of OC levels (from 16.4 ± 4.1 to 33.5 ± 8.7 ng/ml, $P=0.02$). No other differences were observed between the two groups. Increased levels of OC after recovery confirm the inhibitory effect of GC on bone formation.
In CS we found increased levels of OPG maintained after remission of the disease. High serum OPG is also demonstrated in microvascular damage and was associated to an increased cardiovascular mortality. Therefore, high levels of OPG could reflects the persistent damage of the glucocorticoid on bone and cardiovascular system.

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Evaluation of aldosterone to renin concentration ratio as a reliable screening parameter for primary aldosteronism applying currently available assays

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Plasma aldosterone concentration (PAC) to active renin concentration (ARC) ratio (ARR) is an established screening tool for primary aldosteronism. Previously we provided reliable cut-offs measuring PAC by a radioimmunoassay (RIA; Byk&DiaSorin) und ARC by an established immunoluminometric assay (Nichols Institute Diagnostics). Since the latter one is not longer available, we aimed to establish cut-offs for currently available assays.
One hundred seven subjects were studied, including 20 patients with primary aldosteronism (13 adrenal adenomas, 7 adrenal hyperplasia), 15 cortisol-producing adrenal adenomas, 14 pheochromocytomas, 16 nonfunctioning adrenal adenomas, 14 patients with essential hypertension and 34 normotensive volunteers. Patients were on various medications except for aldosterone antagonists. PAC was measured by RIA (Siemens AG), while ARC was determined by an automated chemiluminescent immunometric assay (Liaison, DiaSorin). Putative ratio thresholds were determined by ROC analysis. In 29 subjects, PAC und ARC determined by the current assays were correlated to values obtained by the Byk&DiaSorin/Nichols assay model.
ARR was significantly higher in patients with primary aldosteronism than in the control groups ($P<0.0001$). ROC analysis suggested an ARR threshold of 14.3 (sensitivity 90.0%, specificity 83.9%, AUC$=0.9144$). Additional consideration of PAC >130 ng/l demonstrated a lower sensitivity (80.0%) with higher specificity (95.7%). PAC measured by Siemens was lower ($0.32\times X+34$ ng/l) compared to Byk&DiaSorin, and ARC measured by DiaSorin was higher ($0.96\times X+5$ ng/l) compared to Nichols. Therefore, the current cut-off for ARR is much lower than the previously ones established.
An ARR of >14.3 is a reliable screening tool for primary aldosteronism under random conditions. Application of this assay-specific cut-off will clearly change the diagnosis in a relevant number of patients, compared to previously established ratios.

P50

Metabolic and cardiovascular profile in adult patients with addison's disease under conventional glucocorticoid replacement therapy

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Object
In Addison's disease hydrocortisone or cortisone have so far been used at doses of 30–37.5 mg/day, respectively, though several studies showed that cortisol normal production is about 5.7 mg/m^2 (20–25 mg/day of hydrocortisone or cortisone, respectively). Differently from secondary hypoadrenalism, scanty data exist in patients with Addison's disease on role of conventional glucocorticoid replacement and metabolic and cardiovascular outcome. A recent observational study has reported increase mortality rate in Addison's disease patients conventionally treated.
Design
In 37 Addison's disease patients (11 M and 26 F; 20–71 years) under conventional glucocorticoid replacement therapy (37.5 mg cortisone/day), BMI, fasting glucose and insulin, OGTT, cholesterol and triglycerides (TG), 24 h blood pressure and intima-media thickness (IMT) by eco-doppler ultrasonography were measured and correlated with sex, age, disease's duration, ACTH, PRA and DHEAS.
Results
Mean BMI was in the upper normal range, though higher than 25.0 kg/m^2 in 16 patients. Mean fasting glucose, insulin, HOMA and glucose after OGTT were in the normal range. HOMA was higher than normal in 4 overweight patients and in 2 of them OGTT was diagnostic for IGT. According to ATP III classification, mean total cholesterol was in the desiderable range, none of the patients had HDL lower than 40 mg/dl, whereas LDL was higher than 160 mg/dl in 4 overweight patients; only the 2 IGT patients showed increased TG levels. In all patients 24 h blood pressure showed a normal profile with a preserved circadian variation and IMT was below 0.9 mm. No correlation was found between the above mentioned parameters. None of the patients showed a global CV risk above 5% at 10 years (according to ATP III).
Conclusion
This study suggests that, in Addison's disease, neither conventional glucocorticoid replacement therapy nor the reduced DHEAS secretion are associated with metabolic impairment and/or increased cardiovascular risk.

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Adrenocortical changes and arterial hypertension in lipoatrophic A-ZIP/F-1 mice

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The A-ZIP/F-1 transgenic mouse is a model of lipoatrophic diabetes with severe insulin resistance, hyperglycemia and hyperlipidemia. Recently, a regulatory role of adipose tissue on adrenal gland function and blood pressure has been suggested. To further explore the importance of adipose tissue in the regulation of adrenal function and blood pressure, we studied this mouse model of lipodystrophy. A-ZIP/F-1 mice exhibit significantly elevated systolic and diastolic blood pressure values despite lack of white adipose tissue and its hormones. Furthermore, A-ZIP/F-1 lipoatrophic mice have a significant reduction of adrenal zona glomerulosa, while plasma aldosterone levels and aldosterone synthase mRNA expression remain unchanged. On the other hand, lipoatrophic mice present elevated corticosterone levels but no adrenocortical hyperplasia. Ultrastructural analysis of adrenal gland show significant alterations in adrenocortical cells, with conformational changes of mitochondrial internal membranes and high amounts of liposomes. In conclusion, lipodystrophy in A-ZIP/F-1 mice is associated with hypertension, possibly due to hypercorticosteronemia and/or others metabolic-vascular changes.

P52

Relation between serum adiponectin level, subclinical Cushing's syndrome and non-functional adrenal incidentaloma

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In this study we compared plasma adiponectin levels between patients with non-functional adrenal incidentaloma and subclinical Cushing's syndrome. Our aims were to determine whether there was any difference of plasma adiponectin levels, any relation between cardiovasculer risk factors and to investigate the importance of adiponectin levels in subclinical Cushing's syndrome patients according to non-functional adrenal incidentaloma patients. The patients referred from other clinics to Baskent University Hospital Endocrinology Department due to adrenal incidentaloma were taken to study. In the patients group that was defined as subclinical Cushing's syndrome, the fasting blood insulin and HOMA-IR levels were high and adiponectin levels were distinctively low ($P<0.05$). Although in patients with subclinical Cushing's syndrome relation between adiponectin levels and cardiovasculer risk factors was not found, adiponectin levels were positively related with body fat percentage and HDL-cholesterol, negatively related with waist/hip ratio, body free fat mass and trigliserid levels in group of nonfuntional adrenal incidentaloma. When investigated for HPA axis abnormalities, significant relation between adiponectin levels and cortisol levels after dexamethasone suppression were found. In subclinical Cushing's syndrome patients with adrenal incidentaloma, the low adiponectin levels may be due to glucocorticoid excess independent of metabolic parameters. Plasma adiponectin levels in subclinical Cushing's syndrome patients were no seem to be good marker for determination of cardiovasculer risks and treatment management.

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Subclinical autonomous cortisol hypersecretion by adrenal incidentalomas may be intermittent: results from a long term follow-up study

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Background
Hormonal studies have detected subtle abnormalities of the hypothalamic–pituitary–adrenal (HPA) axis due to autonomous cortisol secretion, in a percentage of patients with adrenal incidentalomas, a condition termed as subclinical Cushing's syndrome. The diagnostic criteria and the clinical significance of this condition are still controversial and consequently there is uncertainty for its management. Data defining the natural course of these tumors, will help to determine clinical implications and appropriate management, but are still inadequate.
Objective
Prospective long-term follow-up study of patients with apparently benign adrenal incidentalomas and without overt hyperfunction at initial diagnosis, in order to assess their hormonal activity and their long-term growth pattern.
Patients-methods
From a group of 89 patients with adrenal incidentalomas, 34 patients with a mass diameter 1.0–6.0 cm (2.2 ± 0.8, median 2.0) were followed-up for 12–144 months, (62.1 ± 33.5, median 57.0), with a hormonal (plasma cortisol rhythm, morning plasma ACTH, serum DHEA-S, serum aldosterone and plasma renine activity, low dose dexamethasone suppression test, 24 h urinary VMA and metanephrines and 24 h urinary free cortisol) and morphological evaluation (CT scan) every 12–24 months. The diagnosis of subclinical Cushing's syndrome was based on a post-LDDST plasma cortisol level >1.8 μg/dl combined with an abnormal result of at least one other test of the HPA axis and the absence of clinical signs of cortisol excess.
Results
At diagnosis 23 patients had a normal adrenal function and 11 had subclinical Cushing's syndrome. During follow-up adrenal function remained normal in 22 patients, subclinical Cushing's syndrome was reassessed in five patients, whilst intermittent subclinical autonomous cortisol hypersecretion was found in seven patients. None of the patients developed Cushing's syndrome. A change in mass size (≥0.5 cm) was found in 12 patients (an increase in nine patients, with no signs of malignancy and a reduction in 3).
Conclusion
These data show that subclinical autonomous cortisol hypersecretion may be intermittent in a significant percentage of patients. This finding supports a wide range of variability from non-functioning adrenal adenoma to autonomous cortisol secreting adrenal adenoma. The spectrum of intensity of subclinical autonomous cortisol secretion may explain the contrasting results of studies

examining long-term adverse implications this disorder. These data also show that a growth tendency is observed in some adrenal incidentalomas, without evidence of malignant transformation.

P54

Preoperative paroxysmal attacks are not predictive of intraoperative hemodynamic changes in patients undergoing laparoscopic adrenalectomy for pheochromocytoma

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Background
Abrupt catecholamine release may cause paroxysmal attacks in patients with pheochromocytoma, and is associated with major hemodynamic changes during adrenalectomy. We investigated whether preoperative paroxysmal attacks are associated with further intraoperative adverse hemodynamic changes during adrenalectomy for pheochromocytoma.
Material and methods
From 1994 to 2006, 88 patients underwent laparoscopic adrenalectomy for pheochromocytoma. In our institution patients are scheduled when normotensiv, and do not receive consistently preoperatively hypotensive drugs unless these drugs have been prescribed elsewhere. Surgery was conducted under standard propofol, sufentanil, tracrium anaesthesia. An arterial line was inserted for arterial pressure (AP) monitoring. Esmolol was administered when heart rate was above 120 mmHg, Nicardipine when systolic AP (SAP) was above 150 mmHg, and Norepinephrine when SAP was <90 mmHg after tumour removal. Paroxysmal attacks were defined as paroxysmal palpitations or elevated heart rate, sweating, flushing, headaches, nausea, or hypertensive access. Whether preoperative paroxysms were associated with esmolol, nicardipine, norepinephrine administration and postoperative morbidity was investigated. χ^2, Mann–Whitney and *t*-tests were used.
Results
Patients of both groups did not differ with respect to demographic data, preoperative hypotensive treatment, intraoperative increased SAP, intraoperative nicardipine, esmolol or norepinephrine use or postoperative morbidity.
Conclusion
Preoperative paroxysms were not predictive of intraoperative hemodynamic changes, thus confirming that there is no evidence-based factors likely to predict intra and postoperative hemodynamic instability, or postoperative morbidity in patients undergoing adrenalectomy for pheochromocytoma.

P55

Adrenal crisis in primary and secondary adrenal insufficiency: frequency and causes

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Adrenal crisis (AC) is a rare but life-threatening complication in chronic adrenal failure. Here we evaluated frequency, causes and potential risk factors of AC in a large sample of patients with primary AI (PAI) or secondary AI (SAI).
In a cross-sectional study 883 patients with AI were contacted by mail. 526 patients agreed to participate and received a disease specific questionnaire. Diagnoses and co-morbidities were verified by review of medical records.
Four hundred and forty four data sets were available for further analysis (PAI $n=254$, SAI $n=190$). 42% (PAI 47%, SAI 35%) reported at least one AC. 384 adrenal crises in 6092 patient years were documented corresponding to a frequency of 6.3 crises/100 patient years (PAI 5.1, SAI 8.2). Precipitating causes were mainly gastrointestinal infection and fever (45% of cases) but also other stressful events (e.g. major pain, surgery, psychic distress, heat, pregnancy) and even sudden unexplained occurrence were reported. Patients with PAI reported significantly more emergency glucocorticoid administrations ($P=0.003$) and AC ($P=0.019$). However, among those patients who had experienced at least one crisis, the frequency of AC was slightly higher in SAI than in PAI (15/100 patient years vs 13.4/100 patient years). Incidence of AC was not influenced by educational status, BMI, glucocorticoid dose, DHEA treatment, age at diagnosis, hypogonadism, hypothyroidism or growth hormone deficiency. In PAI, patients with concomitant non-endocrine disease tended to be at higher risk for AC (RR = 1.24, $P=0.057$). In SAI, female gender (RR = 1.26, $P=0.043$) and diabetes insipidus (RR = 1.25, $P=0.033$) were significantly associated with the incidence of AC.
AC occurs frequently in both PAI and SAI, mainly triggered by infectious disease. However, so far only minor risk factors for AC could be identified in this large cohort, indicating the need of repeated crisis prevention training in all patients with AI.

P56

The role of toll-like receptors in adrenal gland inflammatory response and tumorigenesis

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Sepsis and septic shock remain a major health concern worldwide, and an intact adrenal cortex glucocorticoid (GC) stress response, is critical for organism to survive. Recently, we and others have shown that adrenal gland expresses members of toll-like receptors (TLRs) family which are known to sense pathogens and induce inflammatory response. Data performed on TLRs deficient mice clearly demonstrate a critical involvement of these receptors in immune-neuroendocrine bidirectional crosstalk during infection. TLR-2 and -4 deficient animals developed an impaired GCs response with cytokine dysregulation and ultrastructural impairments of adrenocortical cells. However, the exact mechanism of TLR-mediated adrenal gland dysfunction during bacterial infections is not clear.
Therefore, the aim of this work was to elucidate adrenal gland inflammatory and hormonal response to multiple bacterial-derived ligands, utilising adrenocortical cell line and human cells in primary culture. We found that both human primary adrenocortical cells and NCI-H295R – carcinoma cell line express several TLRs. However, both cell types responded to bacterial-derived ligands differentially. In opposite to NCI-H295R carcinoma cells, adrenocortical cells in primary culture responded to lipopolysaccharide (LPS) and lipoteichonic acid (LTA) stimulation with an enhanced IL-6 and TNF-alpha cytokines and cortisol secretion. Interestingly, the stimulation of adrenocortical carcinoma cells with TLR-1/6 together with TLR-2 ligands (Pam_3Cys_4 or LTA) resulted in a dose and time dependent induction of IL-8, which in turn was not present in non-transformed cells. In conclusion, our data show that TLRs may contribute to adrenal gland dysfunction during systemic or chronic infections by sustaining an excessive inflammatory response. Additionally, TLRs may contribute to the adrenal gland tumorigenesis by inducing of CXC-chemokines secretion such as IL-8, which are found to be overexpressed in adrenocortical carcinomas (ACC) and to contribute to tumour growth.

P57

The sodium to urinary sodium to potassium to urinary potassium (SUSPUP) ratio in primary aldosteronism

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Primary aldosteronism (PA) has a prevalence of about ten percent in a hypertensive population and is an important risk factor for cardiovascular disease. The aldosterone to renin concentration ratio (ARR) is an established diagnostic tool in the screening for PA. However, hormonal determinations are time-consuming and expensive. Therefore, we studied the value of the sodium to urinary sodium to potassium to urinary potassium (SUSPUP) ratio in 37 patients with PA, in 34 hypertensive patients to whom this diagnosis could be excluded (HTN).

The patients groups (PA and HTN) did not differ significantly with respect to sex, age (57.7±1.6 vs 56.3±2.3, respectively), systolic and diastolic blood pressure (means 156 over 93 vs 148 over 88 mmHg). As expected there was a significant difference in the ARR (135.6±98.8 vs 21.7±45.3, respectively) and in plasma aldosterone and renin concentrations. Analysis of the serum and urinary elecrolyte concentrations showed that patients with PA had a significant higher SUSPUP ratios than did patients with HTN (36.9±26.4 vs 16.2±8.5, respectively). Interestingly, treatment with hydrochlorothiazide (HCT) did not interfere significantly with the SUSPUP ratios in PA and HTN.
After spironolactone treatment of patients with PA or operation of aldosterone-producing adenomas the SUSPUP ratios became significantly lower (24.2±11.7) as renin concentrations and blood pressure became normal.
We conclude that the SUSPUP ratio is a cheap tool helpful for the characterization of mineralocorticoid excess syndromes such as PA.

P58

Characterization and differentiation of chromospheres isolated from bovine adrenal medulla

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Chromaffin cells from adrenal medulla are the main source of epinephrine and norepinephrine hormones which are known to mediate so called fight-or-flight response to multiple environmental stress conditions. Chromaffin cells together with sympathetic neurons share the same sympathoadrenal cell lineage, but in contrast to neurons they maintain their proliferation during whole life span. However, little is known about chromaffin cells regeneration in an adult adrenal medulla.
In this study, we focused on isolation and characterization of progenitor cells from an adult bovine adrenal medulla. In the selective conditions, similar to the neuronal progenitors, freshly isolated chromaffin cells in primary culture formed spheres which will be further referred as chromospheres. In addition, chromospheres expressed several progenitor markers including nestin and Sox1. In adult adrenal medulla, adrenocortical cells are in close contact with chromaffin cells. Moreover, DHEA and DHEAS, hormones secreted by zona reticularis, are suggested to influence the adrenal medulla function and regeneration. Therefore, we assessed whether these hormones may directly impact the differentiation potential of cells from chromospheres. Stimulation with DHEAS and BMP-4, factor known to drive neuronal differentiation, induced β-III-tubulin expression suggesting that these factors may promote neuronal fate. In contrary, stimulation with DHEA and dexamethasone induced PNMT mRNA expression which is known to promote chromaffin cells differentiation. Taken together, these data show that cells from choromospheres have differential potential in their response to androgen stimulations which may influence their differentiation.

P59

Evidence for the involvement of endothelial cell products in adrenal CITED2 expression

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The mechanisms that lead to tumor formation of and aldosterone secretion by zona glomerulosa cells in primary aldosteronism are not known. Recently, endothelial cell-derived factors have been characterized that control the release of aldosterone by adrenocortical cells. In addition, we started to characterize the regulation of CITED2, a CBP/p300 interacting transactivator with ED-rich tail 2. Earlier, it was shown in mice that absence of CITED2 leads to adrenal agenesis. Since endothelial cells are in close proximity to adrenocortical cells, we asked whether endothelium-derived factors promote proliferation of adrenocortical cells and – if so – whether CITED2 is involved in this process. We examined the effects of endothelial cell-conditioned medium on aldosterone release by and on the proliferation of adrenocortical cells and on the expression of CITED2, employing the NCI-H295R cell line and primary human adrenocortical cells.
We found a dose-dependent effect of endothelial cell-conditioned medium (ECCM) on wst-1 salt metabolism by and 3H-thymidin incorporation in adrenocortical cells. We found also an increase in the cyclin D3 promoter activity and a significant stimulation of the CITED2 promoter activity which peaked at 500 percent. THese effects were accompanied by an increase in CITED2 messenger RNA and CITED2 protein, as determined by real-time PCR and western blotting, respectively. The stimulatory effect of ECCM could be reversed after silencing CITED2 through siRNAs and by blocking mitogen-activated protein kinase activity using the MEK1-inhibitor PD98059. We conclude that products secreted by endothelial cells are capable in promoting proliferation of adrenocortical cells. This effect was associated with a significant increase in CITED2 gene promoter activity and consequent CITED2 expression. Thus, our data provide evidence that the endothelium regulates factors that are necessary for adrenal organogenesis.

P60

Intraadrenal production of ACTH in macronodular bilateral adrenal hyperplasia causing Cushing's syndrome: its role in the physiopathology of hypercortisolism

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Illicit expression of membrane receptors for circulating regulatory factors, such as GIP and LH receptors, has been well documented in ACTH-independent macronodular adrenal hyperplasias (AIMAHs) causing Cushing's syndrome. In addition, we have observed an abnormal expression of serotonin, arginine vasopressin and ACTH in a subpopulation of steroidogenic cells in two AIMAH tissues. The aim of the present study was: (i) to investigate the presence of ACTH by immunohistochemistry in eleven additional AIMAHs; (ii) to detect ACTH release by the tissue fragments, (iii) to determine whether ACTH secretion can be modulated by activation of illicit receptors, and (iv) to examine the role of local production of ACTH in the control of steroidogenesis.
ACTH-like immunoreactivity was detected in AIMAH tissues, suggesting that this regulatory signal may act through autocrine/paracrine mechanisms to stimulate cortisol secretion. Perifusion experiments demonstrated that adrenal slices spontaneously released detectable amounts of ACTH. In addition, ACTH secretion was significantly increased by GIP and hCG in tissues derived from patients with food-dependent and LH-sensitive hypercortisolism. The ACTH receptor antagonists corticostatin and ACTH (7–38) reduced basal and ACTH-induced cortisol production from the hyperplasia tissues. These data indicate that, in AIMAH tissues, ACTH released by a subpopulation of adrenocortical cells exerts an intraadrenal stimulatory tone on cortisol secretion.

P61

A case of renin-producing adrenocortical carcinoma with Cushing's syndrome: *in vitro* effect of serotonin on renin and cortisol secretions

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A 48-year-old woman was referred for ACTH-independent Cushing's syndrome associated with a left adrenal tumor. Contrasting with hypercortisolism-related hypertension and hypokalemia, renin and aldosterone levels were paradoxically increased. The tumor was surgically removed and pathological examination of the tissue established the diagnosis of corticosurrenaloma. Fragments of the tumor were obtained for *in situ* hybridization, immunohistochemical and cell incubation studies. The presence of prorenin mRNA and renin immunoreactivity was detected in the tumor tissue. Renin and cortisol were detectable in tumor cells culture supernatants whereas aldosterone was not. ACTH and angiotensin II had no action on renin and cortisol secretion. In contrast, 5-HT inhibited renin

production and stimulated cortisol release in a dose-dependent manner. Interestingly, 5-HT-induced cortisol secretion was inhibited by the 5-HT_7 receptor antagonist SB269970 but not by GR113808, an antagonist at the eutopic 5-HT_4 adrenocortical receptor. One month after surgery, UFC and plasma levels of renin normalized. Six months post-operatively, hypercortisolism and hyperreninism recurred concomitantly with hepatic and pulmonary metastatic diffusion of the disease leading rapidly to the death of the patient. Collectively, *in vivo* and *in vitro* data show that the adrenal carcinoma cosynthesized and secreted renin and cortisol. Our results also demonstrate an ectopic expression of 5-HT_7 receptors in the tumor tissue mediating a stimulatory effect of 5-HT on cortisol secretion. In contrast, 5-HT inhibited renin secretion by tumor cells through an unknown receptor type.

Bone and calcium

P62

Recognition of osteoporosis and analysis of influencing factors

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Backgrounds
As the average lifespan in human increases, osteoporosis and osteoporosis-related fractures have been major health care problems. Despite recent advances in medical treatment, few studies have assessed the recognition of osteoporosis by general adults. This study examined the recognition of osteoporsosis and analysed the relating factors.
Methods
We made one to one questions in a rural area called chunjunlee in chunchon city. The questionnaire contained general characteristics of people, sociocultural factors, questions constructed to know recognition of osteoporosis and factors that were presumed to influence people's recognition of osteoporosis. DEXA was done to people who visited medical office of free service.
Results
Total number of people who fulfilled the questionnaire was 204. They were composed of 81 men and 123 women. Women of 83% and of men 72.8% said that they had heard about osteoporosis. There was no significant recognition score difference between men and women. In the items of recognition, wrong answer rate about the association between osteoporosis and musculoskeletal disorders such as osteoarthritis, low back pain was above 90%. Significant factors that influencing recognition were age, education level, menopause state and newspaper/magazine subscription. All men who were diagnosed osteoporosis on DEXA sad that they didn't have osteoporosis.
Conclusions
Many people had conceptual confusion between osteoporosis and other muscular skeletal disorders such as osteoarthritis, low back pain. Physicians played no significant role in improvement of people's recognition of osteoporosis. Generally men thought that osteoporosis was problem of women.

P63

Comparison of ultrasonography and ^{99m}technetium sestamibi in localization of parathyroid adenoma in primary hyperparathyroidism

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Aims
Determine the utility of ultrasonography for the preoperative localization of enlarged parathyroid glands in primary hyperparathyroidism and to compare this method with ^{99m}technetium sestamibi scintigraphy.
Methods
The results of ultrasonographic localization of enlarged parathyroid glands witch were performed by one experienced ultrasonographer were determined in 65 consecutive patients with primary hyperparathyroidism and compared with findings at surgery and results of ^{99m}technetium sestamibi scintigraphy. All patients had biochemically documented primary hyperparathyroidism based on elevated serum calcium and parathyroid hormone.
Results
Ultrasonography detected putative enlarged parathyroid glands in 49 of 65 patients (74.4%) with sensitivity of 83% and positive predictive value of 89%. Sestamibi scintigraphy was positive in 55 patients (84.6%) with sensitivity of 88.7% and positive predictive value of 94.8%. there was no significant deference between ultrasonography and scintigraphy in localization of adenomas. There was a correlation between PTH level and scintigraphic localization of adenoma ($P=0.001$). If both ultrasonography and scintigraphy used for localization, they located 61 adenomas (93.8%) with sensitivity of 93.8% and positive predictive value of 95%.
Conclusion
Ultrasonography is a sensitive and accurate method for preoperative localization of enlarged parathyroid glands in primary hyperparathyroidism, comparable in overall utility with sestamibi scintigraphy. These results suggest that a strategy of initial testing with one or the other method, followed by the alternate imaging test if the first test is negative, would provide correct parathyroid adenoma imaging.

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Daidzein unlike other isolated isoflavones, preserves bone architecture in ovariectomized female rats *in vivo*

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Ovariectomy of immature female rats, results in significant decrease of trabecular bone volume and in cortical bone thickness. Previously we found that estradiol-17β (E_2) restored bone structure of ovariectomized female rats (Ovx) to values obtained in intact sham-operated female rats. E_2 also selectively stimulated creatine kinase specific activity (CK) a hormonal-genomic activity marker. In the present study, we compared the effects of E_2 and the phytoestrogens: daidzein (D), biochainin A (BA), genistein (G), carboxy-derivative of BA (cBA) and the SERM raloxifene (Ral) in Ovx, on both histological changes of bones and CK, when administered in multiple daily injections for 2.5 months. Bone from Ovx rats, showed significant disrupted architecture of the growth plate, with fewer proliferative cells and less chondroblasts. The metaphysis underneath the growth plate, contained less trabeculae but a significant increased number of adipocytes in the bone marrow. D like E_2 and Ral but not G, BA or cBA, restored the morphology of the tibiae, similar to that of control sham-operated animals; the bony trabeculeae observed in the primary spongiosa was thicker, with almost no adipocytes in bone marrow. Ovariectomy resulted also in reduced CK, which in both epiphysis and diaphysis was stimulated by all estrogenic compounds tested. In summary, only D stimulated skeletal tissues growth and differentiation as effectively as E_2 or Ral, suggesting that under our experimental conditions, D is more effective in reversing menopausal changes than any of the other isolated phytoestrogens which cannot be considered as one entity.

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Surgical management of renal hyperparathyroidism: a preliminary series report

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Background
Renal hyperparathyroidism (RHPT) is a frequent complication of uremic patients on hemodialysis and despite various advances in medical therapy parathyroidectomy is necessary in a semnificative number of cases.

Patients and methods
We reviewed our experience (first in Romania) regarding thirtyfour patients with RHPT operated on in our clinic between 1994 and 2007 evaluating the diagnosis methods, surgical indications, techniques and results together with the evolution of our own therapeutical concept. The study included 20 men and 14 women of median age of 48 (range 26–75) years, performing hemodialysis ($n=32$) or peritoneal dialysis ($n=2$) from 8.2 (range 3 to 16) years respectively. Two patients received an unsuccessful renal graft. The diagnosis was established by anamnesis, clinical complaints (mainly osteoarticular pains,osteoporosis, fractures and divulsions), muscle weakness, severe itching and mental troubles), completed by anormal values of calcaemia, hyperphosphataemia and intact PTH. Ultrasonography and CT scan were useful only in 'adenomised' parathyroids and co-existent thyroid pathology.
Results
All the patients were operated on. Twenty subtotal parathyroidectomies 12 total parathyroidectomies (4 with autoimplantation), and two limited resections (adenomectomies) were performed (two video-assisted). There were no deaths and the operative morbidity was 2,9% (vocal cord hemiparesis – one case). Pathology revealed that RHTP was due to four gland diffuse hyperplasia ($n=18$) or nodular hyperplasia ($n=14$).Two neglected parathyroid adenomas with recurrent urolithiasis, finally considered as tertiary HPT, one parathyroid carcinoma (in the fourth parathyroid gland),one thymoma and one occult papillary thyroid carcinoma was identified. Clinical and biochemical cure was achieved at median term control of 38 (range 2–150) months in 85% ($n=29$) of cases.
Conclusion
Parathyroidectomy is effective for long intervals as symptomatic therapy in cases of RHPT appearing in uremic patients on hemodialysis or after renal transplant but the optimal technique must be individualised on each case and still to be debated.

P66

Osteopenia/osteoporosis in adult thalassemic patients: contribution of growth hormone - insulin-like growth factor I deficiency

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GH and IGF-I exert an important role in the control of bone formation. Osteopenia and osteoporosis are a frequent recurrence in patients with thalassemia. Due to the high prevalence of GH deficiency (GHD) in adult thalassemic patients (Scacchi *et al.*, *Clin Endocrinol* 2007), we investigated the possible role of GH - IGF-I abnormalities in the pathogenesis of the osteopenia/osteoporosis of this disease.
Study
Sixty-four adult thalassemic patients (23 men and 41 women, age $31.4+6.8$) were studied. BMD was assessed by DEXA at lumbar spine in 62 patients and at proximal femur in 58. All patients underwent GHRH plus arginine testing. Severe GHD was defined by GH peaks lower than 9 μg/l, partial GHD by GH peaks ranging from 9 to 16.5 μg/l. Blood samples for IGF-I measurement were collected.
Results
Lumbar osteoporosis and osteopenia were demonstrated in 74.1% and 22.5% of patients, respectively. Femoral osteoporosis and osteopenia were documented in 37.9% and 55.1% of patients, respectively. Severe GHD was demonstrated in 25% of patients, while 17.1% displayed partial GHD. IGF-I SDS was low, i.e. below -1.88, in 54.6% of patients. Lumbar *T*-score values were not correlated with either GH peaks or IGF-I SDS values. Femoral *T*-score values were positively correlated with GH peaks ($r=0.38$, $P<0.005$) and IGF-I SDS ($r=0.39$, $P<0.005$). Furthermore, mean femoral *T*-score was significantly lower in patients with severe GHD than in those with normal GH secretion ($-2.94+0.25$ vs $-2.15+0.12$, $P<0.01$).
Conclusions
GH-IGF-I deficiency appears to contribute to bone demineralization of adult thalassemic patients at femural, but not lumbar level. This observation fits in well with experimental and clinical data demonstrating a greater action of GH-IGF-I on cortical rather than trabecular bone.

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Digital X-ray radiogrammetry in followed change of bone mineral density after the treatment in postmenopausal women

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Objectives
To evaluate the effect of alendronate, alfacalcidol, and combinat agent of alendronate and alfacalcidol, in BMD change assess by Digital X-ray Radiogrammetry in postmenopausal women.
Materials and methods
Nine thousand and two hundred and sixty four healthy women with ages between 20 and 89 years referred to our densitometry service using DXR-BMD. Six thousand and six hundred and twelve from these were in postmenopause. Using WHO-criteria for osteoporosis diagnosis, 780 from them presented osteoporosis and 1388 osteopenia. We selected 72 patients who received alendronate 70 mg/weekly; 67 patients who received alendronate 70 mg/weekly and 1 mcg alfacalcidol daily and 30 postmenopausal osteoporotic women who received 1 mcg aflacalcidol daily. Mean age of osteoporotic treated women was 60.9 ± 7.4 years. The treatment was administrated five years and going on. BMD by DXR was measured at each 12 months. Statistical analyses was, *t*-test (Student–Fischer).
Results
In group treated with alendronate the BMD gain after one year was 0.013 ± 0.02 g/cm^2 and 0.031 ± 0.02 g/cm^2 after five years In group treated with combinated agent alendronate and alfacalcidol, BMD gain after one year was 0.019 ± 0.02 g/cm^2 and after five years 0.045 ± 0.02 g/cm^2. In the last group treated by alfacalcidol the gain BMD was 0.009 ± 0.02 g/cm^2 after the first year and 0.020 ± 0.02 g/cm^2 after five years.
Conclusions
The increase of BMD in our group after administration of alfacalcidol was not statistical significantly. Alendronate induced significant increase BMD in postmenopausal women. The increase BMD after alendronate and alfacalcidol was significantly higher than after alendronate only, and demonstrated that the combination is quite effective.

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BsmI, ApaI, TaqI, FokI polymorphisms of vitamin D receptor (VDR) gene and bone mineral density in women with Graves' disease

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Graves' (GD) hyperthyroidism induces accelerated bone turnover that leads to diminished bone mineral density (BMD). Recent studies indicate the associations between *VDR* gene and autoimmune diseases. The role of *VDR* allelic variants in predisposition to primary osteoporosis is also recognized. The authors analyzed if *VDR* polymorphisms: BsmI, ApaI, TaqI and FokI may predispose women with Graves' hyperthyroidism to BMD reduction**.** The subjects were 75 premenopausal female Polish patients with GD aged 23-46 and 100 healthy women. BMD values were evaluated at lumbar spine (L1-L4 region) and femoral neck by dual energy X-ray absorptiometry (DEXA method). The genotyping was performed using restriction fragment length polymorphism (RFLP). The association analysis of VDR polymorphisms and haplotypes with BMD as well as SNPs and haplotypes association with Graves disease were performed. The strong linkage disequilibrium was found for: BsmI, ApaI, TaqI, that formed three the most frequent haplotypes in Graves' women: baT (47.9%), BAt (34.9%), bAT(16.4%). The lowest mean BMD values had homozygotic women with two copies of BAt. However authors did not observe statistically significant association of analyzed haplotypes or polymorphisms with bone mineral density. The analysis of polymorphic variants distribution showed that only presence of F allele of *VDR*-FokI was more frequent in GD women as compared to controls (OR = 1.93; 95% CI:0.97–3.84) with statistical tendency to significance ($P=0.058$).
Conclusions
1. F allele of *VDR*-FokI polymorphism may predispose Polish women to GD.
2. BsmI, ApaI, TaqI, FokI polymorphic variants of *VDR* gene do not predict the risk of decreased bone mineral density induced by excess of thyroid hormones in GD.

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Bone metabolism in hypothalamic obesity

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Obesity protects from osteoporosis. Bone remodeling and energy metabolism could be regulated by the adipocyte-derived hormone, leptin acting on osteoblasts though hypothalamic pathways. Hypothalamic lesions cause obesity. Since most of these patients are also hypopituitary with low IGF1 which could affect bone metabolism the aim of our study was to investigate whether obesity would protect them from osteoporosis.

Three groups of patients were studied: (1) eight hypothalamic obese patients (HO −4 female, mean age 25.5±2.1 years, BMI-38.4±2.8 kg/m^2), (2) seven normal weight hypopituitary (*HN* −4 female, mean age 32.1±2.2 years, BMI-23.8±1.3 kg/m^2) and (3) four diet induced obese patients (DIO-3 female, mean age 27.2±3.1 years, BMI −44.3±3.8 kg/m^2). Patients were sex and age matched. The etiology of obesity in the HO and HN group was due to hypothalamo-pituitary tumor operation or hypothalamo-pituitary disconnection. All patients received standard hormone replacement therapy except for growth hormone replacement after overnight fast serum leptin, IGF1, osteocalcin-OCL, CTx and 25-OH-vitamin D levels were measured. Body composition (Fat%) and bone mineral density (BMD) were estimated by DEXA (Hologic). Relevant parameters are presented as mean±S.E.M. in Table.

Groups	BMI (kg/m^2)	Leptin (ng/ml)	OCL (ng/ml)	CTx (ng/ml)	Vit. D (nmol/l)	IGF 1 (ng/ml)	DEXA *Z* score	BMD (g/m^2)	TBMC (kg)	Fat%
(1) HO	38.4± 2.8	45.5± 4.8	16.2± 2.9	295± 12.6	24.0± 4.0	75.0± 6.0	−0.7± 0.1	0.971± 0.1	2.3± 0.2	43.3± 2.4
(2) HN	23.8± 1.3	–	33.1± 4.2	446± 15.1	–	51.0± 5.7	−1.8± 0.7	0.980± 0.1	–	–
(3) DIO	44.5± 3.8	45.4± 5.0	23.8± 2.4	662± 15.3	65.8± 9.2	164± 8.7*,†	0.4± 0.1†	1.141± 0.1	2.9± 0.2	46.5± 3.1

*$P<0.05$ DIO vs HO; †$P<0.05$ DIO vs HN

Conclusion

1. Obesity induced by hypothalamic lesion with concomitant hypopituitarism (low IGF1), protects from bone loss when compared with normal weight hypopituitary patients with low IGF 1 levels.
2. When compared with diet-induced obesity despite significanlty lower IGF1 levels bone mineral density and biochemical markers of bone turnover were similar.

P70

Birth weight and the relation to lean body mass and BMC in healthy men at peak bone mass: results from the Odense Androgen Study

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Background

Birth weight has been associated with low bone mass in later life. Previous studies, however, have relied on self-reported data on birth weight, included select populations, or been of a limited size. Moreover, it is unclear if the association between birth weight and bone mass is be mediated by body weight, lean body mass, or fat mass.

Aim

We hypothesize that birth weight is associated with peak bone mass in men independent of current lean body mass and body weight.

Participants and design

The Odense Androgen Study is a population-based, prospective, observational study on the inter-relationship between endocrine status, body composition, muscle function, and bone metabolism in young men. In brief, 3000 males aged 20–30 years were randomly selected from the civil registration database in Funen County, Denmark, and invited by mail to participate in the study. Seven hundred and eighty three gave written informed consent to participate in the study and the data are presented here. Bone mass measurements (spine, hip, and whole body) were performed using a hologic-4500a densitometer. Data on birth weight, length at birth, and gestational age was retrieved in a national database covering all birth clinics in Denmark in the current period. The relationship between Birth weight, BMC, Lean body mass and fat mass as tested using multiple regression analysis is shown in the table as partial correlation coefficients.

Results

Data on birth weight and birth length were available on 754 participants. In bivariate analyses, BMC at whole body, spine, and hip were significantly associated with current body weight ($R=0.50$–0.30, $P<0.001$) and birth weight ($R=0.17$–0.09, $P<0.01$). Birth weight was also significantly associated with current body weight ($R=0.09$, $P<0.01$), current lean body mass ($R=0.15$, $P<0.001$) but not current fat mass (*$P<0.05$, **$P<0.01$, ***$P<0.001$).

	BMC		
R values	WB	Spine	Hip
Birth weight	0.08*	–	–
Current fat mass	−0.35***	−0.38***	0.67***
Current lean body mass	0.73***	0.58***	−0.31***
Overall model	0.75***	0.60***	0.67***

Conclusion

Birth weight predicts peak bone mass, but this seems to be due to correlation between birth weight and body weight (and lean body mass) in young adult life.

P71

Every nanogram of 25(OH)D makes a difference: subgroup analysis of institutionalized patients with vitamin D deficiency in Austria

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Elderly residents are at high risk for vitamin D deficiency due to immobilization or little opportunity of uv exposure. Decreased cutaneous capacity to form cholecalciferol and diets low in calcium and vitamin D add to this problem.

In a prospective cohort study that we performed in 95 institutions in four different provinces of Austria we found that 93% of all patients had a 25(OH)D level ≤20 ng/ml. Aim of this study was to analyse whether differences exist in terms of parathyroid hormone and calcium metabolism as well as bone ultrasound measurements at different sites among groups of patients who already have very low levels of vitamin D (<15 ng/ml). Blood samples were collected in the months of May and June in a cohort of 961 female residents above 70 (mean age 84±6 years). Eighty percent of the patients had serum creatinine levels ≤1.2 and the remainder levels ≤1.8 mg/dl. We formed following 25(OH)D groups: 0–4.9 (group A), 5–9.9 (group B), and 10–15 ng/ml (group C). Residents of group A were on average 3 years older and had a slightly less BMI. Multivariate analysis adjusted for age, BMI, mobility status and creatinine clearance demonstrated that group A had significantly lower corrected serum calcium levels (difference 0.07 mmol/l), a 28% increase in serum osteocalcin, a 95% increase in serum PTH levels compared to group C (all $P<0.005$). Quadriceps muscle strength was 12% lower in group A ($P<0.01$) as was the stiffness index at the calcaneus: *Z*-score −0.48 (vs −0.04 in group C, $P<0.005$). Radial and phalangeal SOS measurements were similarly low in all groups.

Our study demonstrates that even within a group of patients with already low 25(OH)D levels patients show significant differences in subgroups of vitamin D deficiency in parameters relating to muscle strength, degree of hyperparathyroidism, bone turnover and bone mass.

P72

Parathyroid hormone and glucocorticoid cooperatively induce RANKL mRNA expression in osteoblasts through different mechanisms

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Glucocorticoid-induced osteoporosis is the most common form of secondary osteoporosis. Glucocorticoid is known to cause hyperparathyroidism due to parathyroid hormone (PTH) secretion. In addition, glucocorticoid and PTH have also been reported to induce RANKL mRNA expression in osteoblasts and promote osteoclastogenesis. However, little is known about cooperative effects of these hormones. The purpose of this study was to clarify the regulation of RANKL mRNA expression by glucocorticoid and PTH in osteoblasts. We used normal osteoblasts and osteoblast-like cell line MG-63. Quantitative real-time RT-PCR revealed that dexamethasone (DEX) and PTH significantly increased RANKL mRNA expression (15-fold, $P<0.05$) for a time-dependent manner. In addition, PTH additively up-regulated DEX-induced RANKL mRNA expression. Moreover, we found that DEX did not influence RANKL transcriptional activity by reporter gene assay using human RANKL promoter. Furthermore, treatment with actinomycin D and DEX markedly prolonged the half-life of RANKL mRNA, as compared to treatment with actinomycin D alone (T1/2=over 24 h vs 10 h), presumably indicating that DEX-induced RANKL mRNA expression is due to the stabilization of RANKL mRNA. In contrast, PTH clearly induced RANKL transactivation and did not influence the stabilization of RANKL mRNA, suggesting that PTH regulates RANKL mRNA expression mainly via RANKL gene transcriptional activation. In conclusion, we showed that RANKL mRNA expression was cooperatively up-regulated by PTH via RANKL transcriptional activation and by glucocorticoid via the stabilization of RANKL mRNA. Thus, glucocorticoid clinically regulates RANKL mRNA expression, at least in part, through two different mechanisms, because glucocorticoid prolongs the half-life of RANKL mRNA directly and activates RANKL gene transcription via glucocorticoid-induced PTH secretion indirectly.

P73

Hyperparathyroidism-jaw tumor syndrome (HPT-JT): a new mutation in the HRPT2-gene

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Inactivating germline mutations in the HRPT2 tumor suppressor gene are the cause of the hyperparathyroidism-jaw tumor syndrome (HPT-JT). The most common feature of HPT-JT is primary hyperparathyroidism, followed by ossifying fibromas of the maxilla and mandible, renal cysts and solid tumors. As recently recognized, mutations of this gene also play a central role in the molecular pathogenesis of parathyroid carcinoma.
A 19-year-old woman presented with a giant cell granuloma of the right mandible which was surgically removed. One year later, she presented with a recurrence of the giant cell granuloma. Serum calcium (3.65 mmol/l) and PTH (398 ng/l) were elevated and the patient was diagnosed for primary hyperparathyroidism. Surgical exploration revealed a parathyroid adenoma on the left side. Postoperatively serum calcium and PTH were within the normal range for the last 10 years.
Mutation analysis revealed the heterozygous mutation c.1423_1433delCT in exon 16 of the HRPT2 gene. This mutation has not been described yet and leads to a shift in the reading frame with a premature stop-codon.
The identification of the c.1423_1433delCT mutation is not only important for the patient herself, but it is also important for other family members who could benefit from the identification as mutation carriers. The patients sister (33 years old) has an encapsulated tumor of the right hip since the age of 12, but has not yet been diagnosed for the HPT-JT syndrome. Early diagnosis can be used for the detection and removal of malignant parathyroid and other associated tumors.

P74

Dramatic hypocalcaemia as a cause of generalized seizures following thyroidectomy

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A 56 years old woman was admitted to an intensive Care Unit for generalized seizure seven days following total thyroidectomy for toxic multinodular goiter. Thyroidectomy included self transplantation of a right parathyroid gland. Pathologic analysis confirmed benign multinodular goiter, without parathyroid tissue. Post operatively, on LT_4 therapy, follow up symptoms included facial paresthesia associated to mild hypocalcaemia (1.56 mmol/l) at day 2, treated with oral calcium (1 g/day) and calcitriol 0.25 μg/day. Symptoms disappeared and the patient was discharged. At day 7, she presented repeated generalized seizure requiring emergency in hospital admission. Blood total calcium concentration was dramatically low, 0.6 mmol/l, associated with low intact PTH concentration (10 ng/l – usual values 10–64). Immediately, calcium gluconate was infused, in conjunction with diazepam and phenitoine. Vitamin D intake was increased, up to 2 μg/day alfacalcidol, resulting in rapid normocalcaemia. Yet, seizure recurred leading to a change in antiepileptic drugs for levetiracetam. A CT scan demonstrated a 20 mm right frontal arachnoidal cyst. The patient remained well and was discharged on LT_4, alfacalcidol and levetiracetam.
Conclusion
This report reminds the rare possibility of seizure in severely hypocalcaemic adult patients, related to cerebral ionic and electric changes. The recurrence of seizure after correction of hypocalcaemia must suggest another convulsive threshold lowering factor, in our report an arachnoidal cyst.

P75

Subclinical hypothyroidism and bone mineral density

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Objective
Hyperthyroidism is accompanied by low bone mass. Because the reference range of TSH levels is defined statistically, some individuals with high-normal TSH levels (TSH <10 mU/l), may have mild hypothyroidism (subclinical hypothyroidism) and reduced bone mass. We therefore determined whether serum TSH levels correlate with bone mineral density (BMD) in persons with subclinical hypothyroidism.
Design
A cross-sectional study.
Participants
Thirty-two postmenopausal women, 18 with hypothyroidism (Hypo) and 14 with subclinical hypothyroidism (Sub Hypo).
Measurements
We measured BMD at the lumbar spine and femoral neck using dual energy X-ray absorptiometry, and serum TSH concentrations using immunoluminometry. Body mass index (BMI) was calculated from body weight and height.
Results
BMD at the lumbar spine (0.816 ± 0.136 vs 0.881 ± 0.106 g/cm^2, P=ns), and total left hip (0.715 ± 0.133 vs 0.673 ± 0.094 g/cm^2, P=ns) didnot differ between the groups. Patients with Hypo had higher level of TSH (7.95 ± 1.51 vs 7.57 ± 2.44, $P=0.03$) and it correlated negatively with BMD at the lumbar spine and total hip. In regression analysis, even after adjustment for age, years since menopause and BMI, subjects with Hypo (TSH$\geq$10 mU/l) have shown significant association between TSH level and BMD at the lumbal spine (Adjusted $R^2=0.82$, $P=0.008$) and femoral neck (Adjusted $R^2=0.78$, $P=0.004$). In the group with Sub Hypo there were no significant association between TSH level and BMD.
Conclusion
These results suggest that high normal TSH level, maybe is not the cause of lower BMD in postmenopausal women, but low number of participants might be the reason of questionable conclusion.

P76

Seasonality, sun exposure, skin type and vitamin D levels influence Parathyroid Hormone related Peptide (PTHrP) metabolism during pregnancy and after delivery in mothers and their newborns

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PTHrP is an important factor for the regulation of calcium homeostasis around delivery. The aim of this study was to longitudinally analyse PTHrP metabolism and influencing factors looking at mothers and their breastfed newborns from 32 weeks of pregnancy until 8 weeks after delivery. Nineteen families (8 Caucasian, 11 Arabic/Asian) participated in the study. We analysed PTHrP, 25 OH vitamin D, parathyroid hormone (PTH), alkaline phosphatase, albumin; serum and urine calcium, phosphate and creatinine. Measures were obtained at 32 weeks of pregnancy (mothers), from cord blood and at 5 days and 8 weeks after delivery (mothers and infants). UVB exposure was continuously quantified by spectral analysis using bio-weighted dosimeters. Surrounding factors and nutrition were assessed by questionnaires and analysis of meteorological data.
Around delivery PTHrP-levels of mothers peaked to a level that was maintained subsequently (pmol/l $\pm$ S.E.M., 32 week/cordblood/day5/8weeks): $0.26 \pm 0.1/0.56 \pm 0.1/0.58 \pm 0.1/\ 0.54 \pm 0.1$, $P < 0.05$. In newborns PTHrP on day 5 decreased compared to cord blood levels and remained low (pmol/l $\pm$ S.E.M., cordblood/day5/8weeks): $0.54 \pm 0.1/0.27 \pm 0.1/0.35 \pm 0.1$, $P < 0.005$. Regression analysis revealed that PTHrP levels between mothers and their children were not correlated. Subjects with mildly pigmented skin (WHO types I and II) had higher PTHrP-levels ($P < 0.005$). PTHrP was positively correlated with sun exposure demonstrating seasonal variations ($P < 0.05$). PTHrP was inversely correlated with 25 OH vitamin D ($P < 0.05$) and not dependent on PTH and calcium levels.
These results reflect that PTHrP is of importance for mothers around delivery and during lactation and for newborns around delivery. It is the first study to demonstrate a longitudinal effect of sun exposure, seasonality, skin type and vitamin D on PTHrP metabolism.

P77

The relationship between bone turnover and body weight and leptin in patients with Anorexia nervosa

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Anorexia nervosa (AN) is associated with bone loss due to weight loss, decreased caloric intake, amenorrhoea and behavioral changes. The most important predictor of the occurrence of bone loss is body weight (BMI < 16.5 kg/m^2). Previous studies suggested that many factors including effects of chronic undernutrition in AN patients is associated with a reduction of bone formation (osteocalcin-OC) uncoupled with increased resorption markers (crosslaps-CTx). The aim of the study was to investigate the relationship of bone turnover and body weight and serum leptin levels in 13 AN patients (BMI 14.3 ± 0.4 kg/m^2; DSM-IV criteria). They were compared with 9 age matched lean controls (BMI 20.1 ± 0.8 kg/m^2; CO). After overnight fast serum leptin, osteocalcin-OCL, CTx and 25-OH-vitamin D levels were measured. Bone mineral density (BMD) were estimated by DEXA (Hologic). Relevant parameters are presented as mean $\pm$ S.E.M. in Table.
Results

Gr	Age (years)	BMI (kg/m^2)	Leptin (ng/ml)	OCL (ng/ml)	CTx (ng/ml)	Vit. D (nmol/l)	DEXA Z score
AN	23.8 ± 1.6	14.3 ± 0.4*	2.3 ± 0.6*	21.8 ± 4.57*	0.7 ± 0.2*	40.4 ± 2.2	-3.2 ± 1.5*
CO	23.3 ± 0.73	20.1 ± 0.8*	9.7 ± 2.1*	29.1 ± 3.18*	0.5 ± 0.1*	38.6 ± 6.8	0.4 ± 0.2*

*$P < 0.05$.

Conclusion
Our results favour the role of body weight, fat mass and leptin in uncoupling of bone turnover with decreased bone formation and increased bone resorption in women with AN leading to decrease in bone mass. The precise mechanism for enhanced bone resorbtion in AN is unclear but is tempting to speculate that leptin might inhibit it.

P78

Usual values and usefulness of crosslaps in pediatric practice

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Serum Crosslaps is a bone resorption specific marker already validated in adults for osteoporosis helping in decision and therapeutic. Not yet clinically evaluated in children, we wanted to analyse the usefulness of this marker in paediatric practice, easier to collect than the other bone resorption markers which need urinary collection often uncertain in children.
Patients and methods
Serum Crosslaps were measured using the One Step ELISA immunoassay (Osteometer) in a population basis of 175 healthy children. The data were compared with results obtained in subgroups of children affected by bone metabolism diseases: osteogenesis imperfecta, mucoviscidosis, hypoparathyroidism, hypercalcemia, corticotherapy, neuro-muscular pathology, precocious puberty, anorexia nervosa.
Results
The paediatric reference data obtained in the population basis showed an important dispersion and significant variations with age and growth: no significant change occurred in either sex until ten years, then there was progressive increase during puberty, peaking at 14–17 years in boys, earlier at 10–14 years in girls, before decreasing again until adults data. There was no significant difference between the results of population basis and subgroups of illness children. However, in cases of children with osteogenesis imperfecta, a decrease of serum crosslaps was observed with bisphosphonate treatment.
Conclusion
Interindividual important changes and variations with age and growth make difficult the use of serum crosslaps in pediatric practice. It could be interesting in individual follow-up of pathology or treatment influencing on bone metabolism, as bisphosphonate treatment in osteogenesis imperfecta. It would be evaluated by carrying on the study on larger samples.

P79

Contribution of vitamin D supplementation to the vitamin D status of infants in the age of fortified milk

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In line with European guidelines, infant formulas in France have been vitamin D-fortified with a vitamin D3 content of $\sim$400 IU/l. New guidelines for the prevention of vitamin D deficiency and rickets have subsequently been published and the vitamin D supplement for formula-fed infants was reduced from 800 to 400 IU per day.
To contribute to the evaluation of the need for vitamin D supplementation, a study was conducted to assess vitamin D3 intake (cholecalciferol) provided by sunlight exposure and fortified milk, and intake provided by supplementation prescribed in form D2 ergocalciferol.
This study was conducted in a population of Caucasian infants.
The vitamin D2 intake, the type and quantity of milk received, time spent outdoors and clothing modalities were recorded.
Vitamin D status was assessed on serum left over from a blood sample obtained during the infant's hospitalization; 25(OH)D2 and 25(OH)D3 levels were evaluated by HPLC followed by radioimmunoassay.
Forty-five term infants between the ages of 2 and 14 months (26 boys and 19 girls) were included, during Winter for 19 cases and during Summer for 26 cases.
The contribution of 25(OH)D2 vitamin D supplementation (mean: 18 ng/ml; range: 2–47) was approximately equal to vitamin D intake from sun exposure and 25(OH)D3-fortified milk (mean: 21 ng/ml; range: 5.5–32).
Three infants had a low $25(OH)D_2$ level and one had a level of 2 ng/ml. The $25(OH)D_3$ level was low (< 10 ng/ml) for 5/36 infants, all included during

Summer.
The most original result of this study is the equivalent contribution to the infant's vitamin D status of D_3-intake by fortified milk and sun exposure and D_2-intake by the prescribed vitamin D supplement. The vitamin D status appeared to be satisfactory under these conditions, with extreme values within the locally defined normal range.

P80

The influence of ESR1 gene genotype on bone mineral density in premenopausal women with Grave's disease

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Introduction
The etiology of this autoimmune disorder results from the presence of stimulating antibodies binding to TSH receptor. The disease has a strong genetic background associated with HLA and CTLA-4 genes.
The aim of study
The aim of this study was to estimate the influence of polymorphic variants of *ESR1* gene on the bone mineral density in young premenopausal women with Grave's disease (GD).
Material and methods
The study was conducted on 75 premenopausal women with GD. The average age of the women was 37 years (from 23 to 46), weight 64 kg (from 51 to 98 kg), height 1.64 m (from 1.50 m to 1.80 m). The analyzed group of women with GD in the state of hyperthyroidism. All of them still had regular menses at the time of this study. The control group consisted of 160 women without thyroid disease
In all of the cases measurements of BMD in the lumbar spine L1-L4 (LS) and femoral neck (FN). Laboratory evaluation included: serum TSH levels, free T_4 and T_3 and TPO thyroperoxidase antibodies levels at the beginning of the study. In all cases there were performed DNA isolation from the peripheral blood and PCR reaction. The PCR product was analyzed by restriction fragment length polymorphism (RFLP) using PvuII and XbaI restrictases.
Results
Homozygotic women with px haplotype had the lowest LS BMD value (1.113 g/cm^2). For this haplotype the tendency dose effect was observed, but not statistically significant.
The dominant effect was evaluated for PX haplotype, homozygotic PX haplotype had the highest LS BMD (1.246 g/cm2). A dose effect for PX haplotype was observed.
In these cases results were statistically significant.
Conclusion
(1) px haplotype of ESR1 gene may determine lower BMD value in the lumbar spine L1-L4.
(2) PX haplotype of ESR1 gene can determine higher BMD value in lumbar spine.

P81

Predictors of bone mineral density in men with primary hyperparathyroidism

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Introduction
In primary hyperparathyroidism (PHPT) bone involvement is frequent. In women with PHPT it has been previously reported that menopausal status together with PTH, calcium levels and renal function are independent predictors of bone density. In male subjects, the impact of PHPT on bone status is less defined.
Aim of the study
To investigate this study was possible predictors of bone mineral density (BMD) in a series of male subjects affected by PHPT.
Subjects and methods
Sixty male patients with PHPT were consecutively studied (age, mean $\pm$ S.D.: 57.9 $\pm$ 14.1 years; Symptomatic/Asymptomatic 32/28; PTH, median and interquartile levels: 119 and 95.0–228 pg/ml, calcium 11.1 $\pm$ 1.0 mg/dl). In all subjects BMI, levels of calcium, PTH, 25 hydroxy-vitamin D, creatinine and creatinine clearance and bone mineral density (BMD) at lumbar spine, femur and distal radius by DXA were evaluated.
Results
A positive association between BMI and DXA measurement at femoral levels was found (BMI-BMD: $R=0.52$, $P<0.004$; BMI-T score: $R=0.53$, $P<0.002$; BMI-Z score: $R=0.55$, $P<0.002$). On the contrary, neither PTH, calcium, renal function nor 25-hydroxy-vitamin D showed significative correlation with BMD levels.
Conclusions
BMI resulted a good predictor of BMD in male patients with PHPT as found in women with PHPT, confirming the important role of BMI as risk factor for bone damage in osteoporosis of both primary and secondary origin. On the contrary, biochemical indices of PHPT as well as renal function did not associate with DXA measures in male patients, differently from female. These findings could indicate gender-dependent differences in the clinical expression of PHPT, particularly at bone level. A peculiar resistance of male bone to PTH excess in PHPT could be also hypothesized. These findings suggest that the clinical management of the modern form of PHPT should be differently by gender.

P82

Primary hyperparathyroidism during pregnancy: a case report

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Primary hyperparathyroidism (PHP) during pregnancy is a very rare event that increases maternal and fetal morbidity and mortality. Complications during pregnancy or neonatal period have included spontaneous abortion, stillbirth, neonatal death, neonatal tetany.
Management of maternal PHP diagnosed during pregnancy should be based on the patients' symptoms and severity of the disease. Hyperparathyroidism characterized by progressive symptoms should be treated surgically, preferably during the second trimester as in the case reported here. A 27-year-old woman was admitted to our clinic at four weeks of gestation with the complaints such as nausea, vomiting and dyspeptic symptoms. One week before, routine biochemical analysis revealed elevated serum calcium level of 13.3 mg/dl and was referred to our clinic. On admission biochemical tests were as follow: Serum calcium 13.7 mg/dl, phosphorus 2.2 mg/dl, albumin 3.6 gr/dl, PTH 109 pg/ml. Ultrasonography of the neck showed a parathyroid adenoma of 1.2×0.5 cm size, in the neighborhood of the inferior pole of the left thyroid lobe. Ultrasonography guided washout was carried out from the suspected adenoma and PTH was measured $>$ 5000 pg/ml within the nodule. After treatment with isotonic sodium chloride, serum calcium dropped to 12 mg/dl and the adenoma was excised in second trimester of the gestation. The postoperative course was uncomplicated. At the discharge from the hospital the patients' serum calcium level was 9 mg/dl. Still she has a healthy ongoing pregnancy.
Hyperthyroidism should be considered and the serum calcium level measured in a pregnant woman who has resistant nausea and vomiting. This condition can be misdiagnosed as hyperemesis gravidarum and underlying disease may be missed. When PHP is discovered during pregnancy, management depends on the degree of the hypercalcemia, gestational age, and presence of complications. Available evidence suggests that the typical mildly hypercalcemic and asymptomatic pregnant woman with PHP can be safely managed conservatively provided neonatal hypocalcemia is sought for and treated. Otherwise, treatment should be surgery preferably in the second trimester of the pregnancy.

P83

Lack of relationship between the BsmI polymorphism in the vitamin D receptor gene and the risk of glucocorticoid-induced osteoporosis in premenopausal asthmatic women on a long-term glucocorticoid therapy

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Results of many studies indicate that the BsmI polymorphism in the vitamin D receptor (VDR) gene may influence bone tissue metabolism and may be useful in identifying patients at greater risk of osteoporosis. As far as long-term

glucocorticoid treatment is a major risk factor for osteoporosis development, we looked for the BsmI VDR gene polymorphism and its relation to phenotypic features characterizing bone status (BMD and metabolic bone turnover) in 74 premenopausal women: 51 asthmatic patients treated for a long-term with GCs and 23 healthy controls. BMD was measured using DXA method. Serum levels of osteocalcin and ICTP (as markers of bone turnover) were measured using IRMA and RIA methods respectively. VDR gene genotypes were determined using PCR-RFLP method. Results: Genotype bb was found in 32.4%, BB in 10.8%, and bB in 56.8%. There were no significant differences regarding BMD, biochemical and hormonal parameters between any of genotypes.
Conclusions
Genotype distribution was similar to those observed in other Caucasian populations. The data suggest that the VDR BsmI polymorphism does not seem to be useful for identifying patients at greater risk of glucocorticoid-induced osteoporosis, however it needs to be confirmed by a population-based study.

P84

Intra-operative and routine intact parathyroid hormone assay on the Beckman Coulter Unicel® DxI 800

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Background
Parathyroid hormone (PTH) assay is critical for diagnosis of calcium disorders and intraoperative (IO) monitoring of parathyroid surgery. We evaluated the new Access® Intact PTH (iPTH) on the UniCel® DxI 800 (Beckman Coulter).
Methods
Imprecision was evaluated for routine and intraoperative (15 min) procedures, at 3 control levels, run 20 times in duplicate over 10 days (NCCLS EP5-A protocol). Normal values were obtained from healthy blood donors (16–66 years; 123 in winter; 163 in summer). Pathological and IO conditions were tested.
Results
For routine assay (iPTH: 30, 291 and 892 pg/ml), intra-assay CVs were 1.9, 1.9, 2.4% and total imprecision was 2.4, 2.6 and 2.4% respectively; for IO procedure (iPTH: 21, 206, 637 pg/ml), intra-assay CVs were 2.0, 2.5, 2.4% and total imprecision 4.4, 4.9, 6.2%, respectively,. iPTH mean normal value was 36 pg/ml (geometrical mean, 95% confidence limits:16–81). iPTH correlated negatively to calcemia ($r=-0.25$; $P<0.001$) and 25-OH vitamin D3 ($r=-0.29$; $P<0.001$). In winter, iPTH level was 10% higher than in summer ($P=0.03$), reflecting differences in vitamin D3. Among 35 surgically proven cases of primary hyperparathyroidism (calcium >10 mg/dl), iPTH ranged from 39 to 252 pg/ml (median: 81). iPTH was lower than 3 pg/ml in 2 children with idiopathic hypocalcemia and in 3 patients with malignancy-associated hypercalcemia. In 18 successful surgical procedures for primary hyperparathyroidism, iPTH concentrations fell by 79% (55%–91%) 10 min after ablation, with no further decrease after 20 min. In 1 case of partial removal of an invading parathyroid tumor, PTH decreased by only 22% after 20 min.
Conclusion
The Access® Intact PTH assay is reliable for diagnostic purposes as well as for intraoperative use, in the setting of a routine core laboratory.

P85

Clinical and hormonal variables to predict bone mineral density loss in anorexia nervosa patients

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Objective
Anorexia nervosa (AN) is often associated with severe mineral bone loss and increased risk of fractures. The objective of this study was to assess the bone mineral density (BMD) in patients with AN and its relationship with different anthropometrics, clinical and hormonal parameters.
Patients and methods
We evaluated 47 women with AN according to criteria of DSM-IV. The anthropometrics variables measured were: weight (kg) height (m), BMI (kg/m^2) and corporal composition. The body fat were measured using a bioelectrical impedance analysis system (TANITA). Hormonal profile: estradiol and IGF-1. The BMD was performed using dual-energy X-ray absorptometry (DXA) in lumbar spine (LS) and femoral neck (FN). WHO criteria were used for defining osteoporosis: T-score ≤ -2.5 S.D. Statistics analysis: the data were evaluated using SPSS 12.0. P value set at 0.05 as a minimal level of significance.
Results
Clinical characteristics: mean age ($\pm$S.D.): 22.2$\pm$3.5 years, BMI 17.7$\pm$2.4 kg/m^2, disease duration 33.3$\pm$9.2 months and amenorrhea duration 17.8$\pm$months.
Sixteen woman (34%) were diagnosed of osteoporosis in LS and 3 (6.5%) in FN.

Duration	BMD Lumbar		T-Score Lumbar		Amenorrhea duration	
	R	*P*	*r*	*P*	*r*	*P*
Weight (kg)	0.41	0.04	0.43	0.03	−0.40	0.08
BMI (kg/m^2)	0.58	0.002	0.58	0.002	−0.31	0.04
Amenorrea duration (months)	−0.44	0.03	−0.45	0.02	–	–
Estradiol (pg/ml)	0.99	0.001	0.99	0.001	0.72	0.001
Body fat (%)	0.57	0.03	0.60	0.01	−0.46	0.01

Conclusion
The women with AN have a decreased BMD in LS and FN, but the lumbar spine is mostly affected by a major risk of fractures. The fat mass loss and the estrogen deficiency are, both, a very important risk factors to the BMD loss, fundamentally to level of LS. The amenorrhea depends fundamentally on nutritional factors.

P86

Vitamin D deficiency and supplementation in pregnancy, a randomised study of 180 women in four ethnic groups

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Objective
To determine the vitamin D status in pregnancy and to evaluate the effects of daily and of single-dose vitamin D supplementation.
Design
A prospective randomised study, in an inner city ante-natal clinic.
Participants
Forty-five women in each of the four ethnic groups, Indian Asians, Middle Eastern, Black and Caucasian, were studied (incomplete delivery data at time of submission).
Intervention
Women were randomised into three treatment groups: a single oral dose of 200,000 IU vitamin D, a daily supplement of 800 IU vitamin D from 27 weeks until delivery and a no treatment group.
Results
At 27 weeks gestation, there was a significantly lower concentration of vitamin D levels in Asian (25+10 nmol/l), Middle Eastern (21+9 nmol/l) and Black (23+13 nmol/l) compared to the Caucasian group (42+17 nmol/l); $P<0.001$. Secondary hyperparathyroidism (PTH levels $>$ 6.8 pmol/l; $n=46$) was significantly higher in Asian (26.7%), Middle Eastern (48.9%) and Black women (24.4%) compared to Caucasian women (2.2%); $P<0.05$. All ethnic groups had normal calcium levels at 27 weeks and at delivery. Significant predictors of Vitamin D levels at 27 weeks are ethnic group, age, parity and daily sunlight exposure greater than 2 hours. There was a significant increase in the maternal vitamin D concentration in the supplemented group (daily 42+12, stat dose 34+13 vs 27+14 nmol/l in the no treatment; $P<0.01$) and cord vitamin D concentration (daily 27+14, stat dose 26+13 vs 17+14 nmol/l in the no treatment; $P<0.0001$). There was no significant difference in the method of supplementation.
Conclusion
Vitamin D levels were significantly lower in Asian, Middle Eastern and Black women compared to Caucasian. Women may potentially benefit from vitamin D supplementation in pregnancy, which can be achieved with a single dose at 27 weeks gestation.

P87

Clinical experience with Cinacalcet for treatment of persistent primary hyperparathyroidism

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Introduction
In primary hyperparathyroidism (PHPT) medical treatment is indicated if the patient donot carry out the surgical criterion, donot accept surgery or there is another serious comorbidity that makes impossible the intervention. Cinacalcet is a calcimimetic that increase the sensitivity of the calcium-sensing receptor (CaSR) to ionized serum calcium, decreasing secretion of PTH and serum calcium concentration. This drug is prescribed for secondary hyperparathyroidism in patients with dialysis and in parathyroid carcinoma. Recent trials suggests it use in the treatment of PHPT.
Objective
To show our experience about the use of Cinacalcet for the treatment of persistent PHPT.
Patients and methods
Three patients (women), age range between 50 and 61 years, diagnosed of persistent PHPT after surgery. The mean time of monitoring was 3 years (range 2–6). Reasons to start Cinacalcet (initial dose 30 mg/12 h): failure of the second surgery, patient 1; gastric cancer and patient's rejection to another surgery, patient 2; serious local fibrosis secondary to previous surgery (thyroid and parathyroid) and patient's rejection to another intervention, patient 3. In two patients previous treatment with biphosphonates failed.
Results
Cinacalcet caused a mean decrease of 2.1 mg/dl (range 1.7–2.8) in serum calcium concentration, without effect in PTH level.
Conclusion
Cinacalcet is an effective alternative in non-surgical treatment of PHPT. We need more studies to know it effects about mineral bone density and quality of life.

P88

R-568 improves transmembrane signal transduction of inactivating mutations of the calcium-sensing receptor

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Inactivating mutations of the calcium-sensing receptor (CaSR) gene, present in homozygous or heterozygous forms, cause neonatal severe hyperparathyroidism or familial hypocalciuric hypercalcemia. The R-568 binds to the transmembrane region of the CaSR thereby enhancing its sensitivity to extracellular calcium ($[Ca^{2+}]_o$) and inhibiting parathyroid hormone (PTH) secretion. The therapeutic potential of calcimimetics like R-568 has been demonstrated in patients with primary hyperparathyroidism as well as in patients exhibiting secondary hyperparathyroidism. In the present study we tested, whether R-568 could improve transmembrane signaling of naturally occurring inactivating mutants of the CaSR.
Mutant cDNAs of seven CaSR mutations (W530G, C568Y, W718X, M734R, L849P, Q926R and Q1005N) were generated and transfected into HEK293 cells. Functional characterization was performed by measuring intracellular calcium in response to varying $[Ca^{2+}]_o$. The transfected HEK293 cells were stimulated with 3 or 10 mM $[Ca^{2+}]_o$ with or without R-568 (1 μM). R-568 significantly enhanced the intracellular Ca^{2+} response to 3 mM $[Ca^{2+}]_o$ in cells expressing the wild-type (WT) CaSR, and the W530G, Q926R and Q1005N mutants ($P<0.01$). The C568Y mutant did not respond to $[Ca^{2+}]_o$ up to 30 mM. In the presence R-568 (1 μM), however, 10 mM $[Ca^{2+}]_o$ caused an increase in intracellular Ca^{2+} by 30 nM ($n=10$). By contrast, in cells expressing the W718X, M734R, and L849P mutant no $[Ca^{2+}]_o$-induced cytosolic Ca^{2+} response could be observed even in the presence of R-568 (1 μM).
In conclusion, the allosteric calcimimetic R-568 could enhance transmembrane signaling in certain inactivating mutants of the CaSR. This might offer medical treatment to severely affected individuals (homozygotes) harbouring a calcimimetic-sensitive CaSR mutant. The sensitizing action of R-568 appears to be restricted to mutants, that have mutations in the extra- or intracellular part of the CaSR, since all non-responsive mutants were either truncated (W718X) or mutated in the transmembrane domain (M734R, L849P).

P89

Relationship between parity and osteoporotic fractures in postmenopausal osteoporotic women (PMOW)

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Several studies have shown a positive correlation between parity and reduced hip fracture rate later in menopause while others have shown no correlation or a negative one. Thus, it is possible that some of the conflicting results reported in literature may be attributable to differences in the ages of the populations studied or of the concomitant presence or not of osteoporosis.
In order to elucidate these conflicting results, a population based observational retrospective study has been performed at 160 centers all over Greece. The fracture rate of 4616 postmenopausal osteoporotic women (PMOW) (mean age $=64.1\pm9.3$ years) has been compared with the number of their children. Patients have been divided into two main groups. Group A consisted of women with two or less children and Group B of women with 3 and more children. Descriptive statistics like the mean $\pm$ S.D. and frequencies were used to present the data. In order to assess for relationships between categorical variables the χ^2 test was performed. Statistical analysis was conducted using the software SAS, version 9.1 and statistical significance was established as 5%. The results are as follow:
1. 16.2% of these PMOW had a history of fracture and for 80.3% of them was a hip fracture.
2. There was no correlation of the parity in general and the rate of fracture ($P=0.6887$).
3. Group B women had higher fracture rate than Group A women which was a statistically significant result ($P<0.05$).
It can be concluded that more fracture-susceptible PMOW are those having three children or more. It seems that the beneficial effect of multiparity on BMD, as it has been described by others, may be lost after menopause especially in an osteoporotic population.

P90

Relationship between osteoporosis and living/working environment

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Osteoporosis can be caused by many miscellaneous factors. These factors include medical, lifestyle and socioeconomic variables, the latest being not well studied and defined in international bibliography. From these there are the factors regarding the working environment (house or office) and the living environment (urban or countryside). Our hypothesis is based on the fact that women living in an urban environment or working in an office environment should have lower Bone Mineral Density (BMD) and thus, greater fracture possibility because of their lower level of physical activity, greater alcohol/coffee consumption and increased smoking frequency compared to women living in the countryside or women housekeeping.
In order to find whether this hypothesis is true, a population based observational retrospective study has been performed. The fracture rate of 4616 postmenopausal osteoporotic women (PMOW; mean age $=64.1\pm9.3$ years) from 160 centers all over Greece has been compared with the two aforementioned possible risk factors. Descriptive statistics like the mean $\pm$ S.D. and frequencies were used to present the data. In order to assess for relationships between categorical variables the χ^2 test was performed. Statistical analysis was conducted using the software SAS, version 9.1 and statistical significance was established as 5%.
The results are as follow: (1) 16.2% of these PMOW had a history of fracture and for 80.3% of them was a hip fracture (2) 84.1% of PMOW lived in urban environment and had lower fracture rate than women living in the countryside ($P<0.05$) and 3) 47.2% of the PMOW worked at home and had lower fracture rate than women working for more than 20 years in an office environment ($P<0.0001$).
It can be concluded that more fracture-susceptible PMOW are those working in an office environment and also living in the countryside. It can be assumed that the first is related with lower BMD and the second with the more 'fall-prone' nature of the country environment.

P91

Determinants of spinal deformities in adult patients with untreated growth hormone deficiency (GHD)

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Adult GHD patients may have reduced BMD with high risk of vertebral and non-vertebral fractures which is thought to be reverted by long-term rhGH replacement therapy. In this study we aimed at identifying the determinant factors of vertebral fractures, as assessed by a radiological morphometric approach, in a cohort of adult patients with untreated GHD. Forty-two patients (27 males, 15 females; median age: 48 years, range: 30–67) with untreated severe (as defined by a peak GH response to a stimulation test of <3 μg/l) GHD were evaluated for vertebral deformities (T4-L5 quantitative morphometric analysis according to Genant score) and bone mineral density (dual-energy X-ray absorptiometry). Radiological vertebral fractures were found in 33 patients (78.6%). Twenty-two patients had two or more fractures, whereas in 11 patients the fractures were single. Moreover, the fractures were mild in 54.8%, moderate in 19.0% and severe in 7.1% of patients. Fractured patients had a duration of GHD significantly longer as compared with the patients who were found without vertebral fractures (10.4 ± 1.9 vs 3.4 ± 0.9 years; $P=0.002$). The duration of GHD was also correlated with the number of fractures. Fractured and non-fractured patients showed not significant differences in age, sex, bone mineral density, prevalence of untreated hypogonadism. Moreover, fractured and non-fractured patients showed comparable serum IGF-I values (79.3 ± 6.9 ng/ml vs 89.7 ± 12.3 ng/ml). However, serum IGF-I values were correlated with the number of vertebral fractures, independently of the duration of GHD. In fact, in the patients with lower serum IGF-I values (1st tertile) the prevalence of multiple spinal fractures was significantly greater as compared to patients with higher IGF-I values (3rd tertile; 76.9% vs 33.3%; $P=0.01$), although the two groups of patients did not show significant differences in the overall prevalence of vertebral deformities (86.7% vs 73.3%). Furthermore, the patients with severe spinal fractures showed significantly lower IGF-I values as compared to patients with mild spinal deformities (39.7 ± 15 ng/ml vs 90 ± 7.6 ng/ml; $P=0.009$). In conclusion, in adult patients with untreated GHD the duration of disease seems to be the main predictor of vertebral fracture risk. However, patients with lower serum IGF-I levels are those at risk of a more severe bone impairment.

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Osteoprotegerin and bone mineral density in adult patients with celiac disease

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Background
Calcium and vitamin D malabsorption in celiac disease (CD) predispose to high bone turnover and skeletal demineralization. Osteoprotegerin (OPG), a member of the tumor necrosis factor receptor family, and ligand of receptor activator of NFkB (RANKL) inhibits osteoclast formation and activity.
The aim of this study was to evaluate the relationship between bone mineral density (BMD), bone-turnover markers and osteprotegerin in adult patients with CD and assess whether a gluten-free diet (GFD), calcium and vitamin D supplementation are sufficiently effective for BMD restoration.
Methods
BMD, calcemia, calciuria, and serum osteoprotegerin, Vitamin D, parathormone (PTH) and bone-turnover markers (ALP, osteocalcin, ICTP (C-terminal telopeptide of type I collagen)) concentrations were measured in 27adult CD patients and in 26 controls. Then the CD patients were treated with a GFD and calcium plus alfacalcidol for one year.
Results
Reduced BMD was diagnosed in 57–77% of the patients. Mean calcemia, calciuria, and 25(OH) Vitamin D were lower, but serum PTH and bone-turnover markers were significantly higher in CD patients than in controls. After one year of treatment, the biochemical abnormalities were considerably reduced, and BMD significantly increased ($P<0.05$), mostly in the lumbar spine.
Serum osteoprotegerin concentration was higher in CD patients than in controls before ($P=0.001$) and after ($P=0.009$) treatment. There was no correlation between OPG, PTH and bone turnover markers in CD patients. The negative correlation was observed only between OPG and osteocalcin in controls and in a group of CD patients after treatment. The slight negative correlation was found between OPG and spine BMD in whole examined group ($P<0.05$).
Conclusions
The OPG is involved in the process of bone turnover in celiac disease, but the mechanism is not clear. The elevation of OPG might be a compensatory mechanism against other factors that promote bone damage, but perhaps it is a part of inflammatory process in CD.

P93

Insulin sensitivity and lipid status before and after radical treatment of primary hyperparathyroidism

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It was previously shown that patients with primary hyperparathyroidism (PHPT) are insulin resistant. The aim of our study was to evaluate the effect of surgical treatment on insulin sensitivity and lipid levels in patients with PHPT. In 21 patients with PHPT (age: 56.24 ± 8.69 years, BMI 25.2 ± 3.81 kg/m^2) lipid levels (TC, HDL, LDL, TG, Lp(a)) and insulin sensitivity were determinated before and 4 months after surgical treatment. Insulin sensitivity was evaluated using euglycemic hyperinsulinemic clamp.
Results
There was significant difference in insulin sensitivity (M index: 4.07 ± 2.05 vs 7.14 ± 4.91, $P<0.01$) before and after surgical treatment, while there was no change in BMI before and after testing (25.20 ± 3.81 vs 25.39 ± 3.56, $P>0.05$). There was no difference in TC (6.07 ± 1.45 vs 5.97 ± 1.35, $P>0.05$), HDL (1.23 ± 0.35 vs. 1.25 ± 0.36, $P>0.05$), LDL (3.85 ± 1.18 vs 3.93 ± 1.26, $P>0.05$), Lp(a) (0.21 ± 0.27 vs 0.18 ± 0.22, $P>0.05$) and TG (1.87 ± 0.81 vs 2.16 ± 1.03, $P>0.05$) levels before and after surgical treatment. In conclusion, radical treatment improves insulin sensitivity in patients with PHPT.

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Scintigraphic, biochemical and clinical response to zoledronic acid treatment in patients with Paget's disease of bone

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Introduction
Bisphosponates have long been used with success in the treatment of Paget's disease of bone (PDB). The aim of this study was to evaluate the early (up to three months) and late (at twelve months) scintigraphic, biochemical and clinical response to a single intravenous infusion of zoledronic acid (ZOL) in patients with PDB serially assessed for one year.
Materials and methods
Nine patients with 30 bone lesions due to PDB were prospectively evaluated. Total serum alkaline phosphatase (SAP) was serially measured. Scintigraphy was performed before, three and twelve months after ZOL administration and bone lesions were assessed quantitatively.
Results
After treatment, pain was alleviated in five out of six patients starting from the first month. At three months, a significant decrease of SAP levels compared to baseline values was found (322 ± 211 IU/l before versus 101 ± 36 IU/l three months after, $P<0.05$), with normal values attained in all but one patient. The scintigraphic index of involvement (SII), a marker for the per-patient activity of the disease, was reduced from 14.4 ± 7.6 to 7.2 ± 1.8 ($P=0.01$). The scintigraphic ratio (SR), a marker for the per-lesion activity of the disease, was reduced from 12.8 ± 7.7 to 7.0 ± 2.9 ($P<0.001$). The values of markers of disease activity remained unchanged up to twelve months.
Conclusion
A single intravenous administration of ZOL leads to a favorable clinical, biochemical and scintigraphic response in patients with PDB starting as early as three months after treatment and lasting no <12 months (i.e considerably longer than the existing other therapies).

P95

Significance of bone mineral density measurement at multiple skeleton sites

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Site-discordance in bone mineral density (BMD) assessment is common and affects patient categorization. Greater number of osteoporotic sites correlates with lower *T* score at each index site. The effect of BMD measurement at the contralateral femur on osteoporosis diagnosis has been previously evaluated.
The aim of the study was to investigate the effect of BMD measurement at the contralateral femur on osteoporosis diagnosis.
Methods
BMD of the lumbar spine and both femurs was measured by dual energy X-ray absorptiometry in 93 consecutive Caucasian women aged ≥ 50 years (62.4 ± 8.7 (mean $\pm$ S.D.) years) with a body mass index of 29.2 ± 4.5 (range 20.8–45.2). Using the *T* score from each site, measurements were classified according to the WHO criteria for the diagnosis of osteoporosis as normal, if *T* score > -1, osteopenia, if *T* score < -1 and osteoporosis, if *T* score < -2.5. The results from the three skeleton sites were compared to each other using the statistical package SPSS and the effect of the inclusion of the contralateral femur on the diagnosis of osteoporosis was determined.
Results
BMD did not differ between the two femurs ($P > 0.05$, paired Student's *t*-test). In 77 (82.8%) of the women the classification would be the same if either two or three sites were measured. In 16 (17.2%) of the women the classification would be affected by the measurement of the contralateral femur. In 8 (8.6%) of them *T* score of the lumbar spine was equal or lower than that of both femurs, thus lumbar spine measurement determined the diagnosis. In the remaining 8 (8.6%) classification depended on the choice of the femur measured and would be different if only the right or left femur was measured.
Conclusions
BMD measurement was not different between the femurs. Although the number of cases included is small, it appears that determining BMD at the lumbar spine and one femur is enough for a correct evaluation and diagnosis in the majority of the patients (90%). Nevertheless, in a small number of patients the choice of the femur measured may affect patient diagnosis and classification.

P96

Effects on bone turnover and bone mineral density of levothyroxine suppressive therapy in patients with benign nodular goiter

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Introduction
Thyroid hormones play an important role in bone metabolism. The potential action of prolonged levothyroxine therapy on bone mass reduction is still a matter of debate.
Objective
The aim of the present study was to assess the effects of levothyroxine treatment on bone mineral density (BMD) and biochemical bone turnover markers.
Methods
We determined thyroid function – TSH and free T_4 (FT_4), regional BMD parameters (lumbar spine, femoral neck, trochanter and Ward's triangle) and biochemical bone turnover markers (serum calcium, parathyroid hormone, alkaline phosphatase and osteocalcin and urinary deoxypyridinoline) in 58 women with benign nodular goiter. Forty one were submitted to suppressive levothyroxine therapy (group A) and seventeen remained without therapy for an identical period (group B). We performed initial and final biochemical and densitometric evaluations and compared variations between groups, for all parameters.
Results
The population studied was characterized by a mean age of 51 ± 13 years and TSH 1.77 ± 1.3 μUI/ml. None of the patients had initial osteopenia/osteoporosis or alterations in biochemical evaluation. The mean daily dose of levothyroxine was 97.5 ± 32.8 μg and the mean duration of treatment was 10.3 ± 6.8 months (mean cumulative dose of 30.8 ± 24.3 mg). Considering all patients, there was a significant reduction in TSH ($P = 0.000$) and an increase in FT_4 ($P = 0.001$), alkaline phosphatase ($P = 0.004$) and deoxypyridinoline ($P = 0.029$) at the end of the study. Those variations were significantly more pronounced in group A ($P = 0.000$, $P = 0.002$, $P = 0.003$ and $P = 0.02$, respectively). There was no significant difference in BMD parameters between groups.
Conclusions
Our data suggest that levothyroxine suppressive therapy raises bone turnover. However, medium-term therapy does not seem to induce an increase in bone demineralization. It would be necessary to amplify the evaluation period to fully exclude a deleterious effect of levothyroxine therapy on BMD.

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A new form of hereditary low-turnover osteoporosis in a 3-generation Finnish family

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Juvenile primary osteoporosis, unless diagnosed as osteogenesis imperfecta, has previously been considered a sporadic and self-limiting disease. New genetic findings, including osteoporosis-causing mutations in the LDL-receptor related protein (LRP) 5 and LRP6 genes, challenge this view. The pathogenesis of juvenile osteoporosis still remains largely unknown. We describe findings in a three-generation pedigree with a new form of autosomal dominant osteoporosis.
The proband, at age 35, presented with multiple painful thoracic vertebral compression fractures causing a loss of 7 cm of her adult height. Secondary causes of osteoporosis were excluded. She has no features of OI and no peripheral fractures. The lumbar bone mineral density (BMD) *Z*-score is -2.9. Assessment of the pedigree revealed osteoporosis in eight additional family members, many with asymptomatic compression fractures. The youngest is a boy of 12 years with asymptomatic compression fractures in his thoracic spine and a lumbar BMD *Z*-score of -1.7. Transilial bone biopsies from two treatment-naïve adult family members revealed severe low-turnover osteoporosis with low trabecular bone volume, decreased osteoid and low numbers of osteoblasts. Mineralization and resorption rates were normal. A genome-wide micro-satellite analysis yielded a maximum lod score of 2.8 and further analyses are under way to identify the disease-causing genetic defect. No linkage was found in areas encoding type 1 collagen. Genetic testing for mutations in LRP5 and LRP6 was negative.
The described three-generation family represents a new form of autosomal dominant early onset primary osteoporosis. The discovery of the underlying genetic defect may provide important new information about the biological and pathogenetic mechanisms of osteoporosis.

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***In utero* and postnatal exposure to phytoestrogens modulates the bone mineral density of juvenile and adult female wistar rats**

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Effects of phytoestrogens on female estrogen sensitive tissues are discussed controversially. There is growing evidence that exposure early in life may have a larger (and lasting) impact than an exposure later in life.
To further investigate this issue we performed a study in female Wistar rats. Animals were divided into three groups: Group K received a phytoestrogen free diet, group P phytoestrogen rich diet and G a phytoestrogen free diet supplemented with genistein (Gen, daily intake 100 mg Gen/kg per BW) prior to mating, throughout pregnancy and up to weaning. Their offspring were kept on the diets during growth and then sacrificed at the age of 25, 50 or 80 days.
In groups G and P significant increases of the Gen plasma levels were detected. At day 25, the uterine wet weight, the height of the uterine epithelium and the expression of complement C3 were significantly stimulated in group G animals compared to the K and P group. At day 80 no significant differences were

observed between the treatment groups and the control group. Interestingly, at day 50 the uterine wet weight was significantly increased in groups G and P. The trabecular bone mineral (BMD) density of the groups G, but also P was significantly elevated compared to group K, both in juvenile rats (at day 25) and in adult rats (at day 80).
In summary, our results demonstrate that exposure to phytoestrogens during gestation, lactation and growth increases the BMD of juvenile but also adult female rats. Interestingly, a phytoestrogen rich diet results in an increase of BMD but in contrast to the administration of GEN, does not stimulate estrogen sensitive uterine parameters. These findings support the hypothesis that lifelong exposure to phytoestrogen by diet may be responsible for the different epidemiology of fracture risks in eastern and western countries and may protect against osteoporosis.

P99

Secondary osteoporosis in man with aromatase deficiency: positives and negatives of estrogen therapy

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To date only seven cases of naturally occuring inactivating mutations of the aromatase gene in men have been documented. Osteoporosis is one of the typical signs of the aromatase deficiency in male patients. We report results of estrogen therapy in patient with formerly described frameshift mutation of the *CYP19* gene, ins 1058 T. Bone mineral density (BMD) was assessed during three phases of estrogen therapy. Patient was substituted by calcium and vitamin D throughout the study. Therapy started with t.d. estradiol (E_2) at dose 50 μg daily two days in week (phase 1). BMD increased in all measured localisations (vertebral +16%, neck +5.5%, ultradist +11%). Due to mild increase of body weight (+3.6 kg) as well as worsening of insulin resistance (fasting insulinemia 167.0 mIU/l opposed to 27.5 mIU/l in phase 1) we reduced dose of t.d. E_2 to 25 μg daily one day in week (phase 2). After this change, fasting insulinemia moderated to 58.9 mIU/l, however we registered fall of BMD in lumbar spine (vertebral −9%, neck +14%, ultradist +11%). Therefore dose of E_2 was increased and considering the patient's preference we started equivalent i.n. formulation of E_2 – 2 injections of E_2 hemihydrate (1 injection ≈ 0.07 ml contains 150 μg of E_2 hemihydrate) weekly, i.e. 300 μg weekly equivalent to t.d. E_2 in dose 50 μg daily one day in week. At this dose we noted divergent changes of BMD (assessment after phase 3, vertebral +8%, neck −7%, ultradist −13%). Although showing positive effect to BMD, general effect of E_2 therapy (depending on dose, length, formulation) demonstrating as worsening of *a priori* presented metabolic syndrome, remains questionable.

P100

Agressive course of primary hyperparathyroidism caused by parathyroid carcinoma: case report

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Parathyroid carcinoma (PTCa) is an uncommon cause of primary hyperparathyroidism (PHP), statistically representing <1–2% of all cases of PHP. Early resection of the primary tumor is the only curative treatment, however this is frequently incomplete. As a consequence, recurrence of PTCa presenting as locally invasive tumor and/or metastatic process is not rare. Morbidity and mortality are generally caused by the effects of unremitting hypercalcemia rather than tumor growth.
We present our own experience with management of the patient with PTCa. After the diagnosis of PHP was established, during two months patient underwent two unsuccessful surgeries (without histological confirmation of parathyroid tissue. Severe hypercalcemia 4.47 mmol/l with PTH-I level 600 pg/ml (reference value < 68.3) was reason for urgent admission one month later. MRI showed pathological jugular mass with enlargement of supraclavicular, submandibular and paracarotid lymph nodes. Tumoral mass was only partially removed. PTCa with infiltration of the thymic tissue was confirmed histologically. PET showed residual hypermetabolic mass (with latero-lateral distance 3.1 cm) and multiple bilateral lung metastases (14 in right lungs, 12 in left lungs). Due to the metabolic instability and size of the tumor residuum, patient was not appropriate candidate for immunization with parathormone fragments – novel and perspective modality in medical therapy of PTCa. Worsening clinical course of the patient and progression of lung metastases unabled repeated sugery. Rapid increase of calcemia to 5.60 mmol/l required acute hemodialysis and consequent hemodialyses in every other day intervals. Despite multinodal medical therapy – increased fluid intake, loop diuretics, parenteral bisphonates (pamidronate and zolendronate) and calcimimetics (cinacalcet) and finally hemodialysis, patient died after 12-month duration of the disease.
We are awaiting the results of molecular analysis of *HRPT2* gene. The identification of a mutation would be valuable as question remains whether genetic testing of *HRPT2* gene should be offered to and performed in all patients with PTCa and in all at-risk relatives of a patient who has PTCa with an *HRPT2* germ-line mutation. Monitoring serum calcium levels in all persons at risk provides an alternative to definitive DNA diagnosis.

P101

FGF-23 and parameters of calcium and bone metabolism are positively influenced by GH replacement in adult growth hormone deficiency patients from the KIMS survey

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Growth hormone deficiency leads to reduced bone turnover, mainly due to alterations in PTH circadian rhythmicity as well as to a lack of sensitivity of the kidney and bone to the effects of PTH. These mechanisms may be responsible for bone turnover changes and development of osteoporosis. Patients with GHD show abnormalities of renal phosphate metabolism, which may also contribute to the pathogenesis of bone loss in these patients. GH treatment leads to restoration of renal tubular reabsorption and sensitivity of target organs to PTH, bone turnover and improvement of BMD under GH replacement therapy.
The role of the phosphatonine FGF-23 in this complex process was investigated. We measured the relationship between parameters of calcium metabolism such as Calcium, Phosphate, Creatinine and PTH and their correlation to the phosphatonine FGF-23 and 25-OH Vitamin D levels in GDH patients before, after 6 and 12 months therapy with growth hormone.
EDTA-Plasma (due to better stability than serum samples) of 15 patients was investigated. Arithmetic means for intact FGF-23 before therapy were 17.3 pg/ml, after 6 months therapy 15.3 pg/ml and 23 pg/ml at 12 months (all $P<0.001$). C-terminal FGF-23 initial was 58 U/ml, at 6 months 47 U/ml and at month 12 108.6 U/ml (all $P<0.001$). Calcium values were 2.38, 2.44 and 2.43 mmol/l. Corresponding phosphate levels were 3.04, 3.39 and 3.08 mg/dl (all $P<0.001$). As expected, FGF-23 was inversely correlated to serum phosphate.
GH supplementation in GHD patients leads to normalization of tubular reabsorption of phosphate due to regulation by FGF-23 and reverses the GHD-induced relative phosphate-deficient state. Restoration of the physiological regulation of phosphate metabolism emphazises the beneficial effect of GH supplementation on bone remodeling in GHD patients.

P102

Endogeneous calciotropic and sex hormones and their relationship to bone mineral density in elderly females

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Purpose
To determine the effect of endogenous calciotropic and sex hormone concentration on bone parameters in postmenopausal women.

Methods
We analyzed baseline data of the Senior Fitness and Prevention Study (SEFIP), an 18-month randomized controlled exercise trail with 246 females, 65 years and older. Baseline assessments of Bone Mineral Density at the lumbar spine (LS) and proximal femur were performed using DXA technique. Blood samples were taken from each participant after an overnight fast between 0700 and 0900 h. Serum-estradiol, testosterone, SHBG, DHEA-S, vitamin-D (25OHD) and PTH (Roche Diagnostics, Mannheim, Germany) were determined. For each of these independent parameter our cohort was grouped into terziles with the same numbers of subjects according to the hormonal concentration. Here, we focus on the comparison of spinal and femoral BMD between the highest (1) vs the lowest terzile (3).
Results
Significant ($P<0.01$) between group-differences in respect to the BMD at LS and hip were observed for Estradiol (1>3), SHBG (1<3) and the Estradiol/SHBG fraction (1>3). Although there was a tendency for higher BMD-values in the highest terzile (1) significance could not be reached for Testosterone, DHEA-S and 25OHD. No difference at all was determined for PTH.
Conclusion
Estradiol, SHBG and 'bioavailable' Estradiol clearly predict BMD at the lumbar spine and femoral neck in postmenopausal females 65 years and older.

P103

Exercise effect on bone anabolic hormones in elderly females: preliminary results of the senior fitness and prevention study (SEFIP)
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Purpose
To determine the effect of a combined ambulatory exercise program on sex steroid concentration in postmenopausal females.
Methods
The Senior Fitness and Prevention Study (SEFIP) is an 18-month randomized controlled exercise trail with 246 females, 65 years and older. The exercise group (EG) performed a vigorous endurance, strength and balance training two times per week, the 'wellness control group' (WG) performed a low intensity and low volume session once per week for 4×10 weeks during the 18 months. Both groups were adequately supplemented with calcium and vitamin D. Serum-Estradiol, testosterone, SHBG, DHEA-S, vitamin D (25OHD) and PTH (Roche Diagnostics, Mannheim, Germany) were determined twice, before and after the intervention always between 0700 and 0900 h after an overnight fast.
Results
After 18 months samples were available for 110 women of the EG, and 114 subjects of the WG. Although a tendency for more favorable changes in the exercise group were determined for 25OHD, PTH, DHEAS and Testosterone, significant between-group differences could only be observed for Estradiol ($+6.1\pm15.7$ vs $-1.7\pm13.6\%$, ES) and 'bioavailable' Estradiol (E2/SHBG).
Conclusion
Our results indicate that besides mechanical pathways high intensity exercise programs may impact bone via changes of bone anabolic hormones.

Clinical cases

P104

A case of pseudopseudohypoparathyroidism with vitamin D deficiency
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We describe below a 72-year-old woman who presented clinically with short metacarpals and metatarsals and the following blood biochemistry: corrected calcium of 2.16 mmol/l (normal: 2.05–2.60), phosphate 0.61 mmol/l (normal: 0.8–1.45) and alkaline phosphatase of 108 U/l (normal: 35–104). She was 153 cm tall and weighed 109 kg with a round face. She had a history of Monoclonal Gammopathy of unknown significance, vitamin B12 deficiency and was admitted 10 years ago with a corrected calcium of 1.72 mmol/l and has been on alfacalcidol (1α-hydroxycholecalciferol) since then. Her current medications included alfacalcidol 1 μg on alternate days and vitamin B12 injection 1 mg every 3 months. On enquiry she informed that all her three siblings and one son have brachydactyly with none having low calcium. Further investigations showed a normal serum magnesium and a high parathyroid hormone of 7.6 pmol/l (normal: 1.1–6.9) with an ionised calcium of 1.07 mmol/l (normal: 1.05–1.30) and a phosphate of 0.64 mmol/l. Despite having the morphological features of Albright's osteodystrophy a low phosphate was inconsistent with the diagnosis of pseudohypoparathyroidism type 1a. There was no provision for Ellsworth Howard test. A vitamin D level requested revealed severe vitamin D_3 deficiency (5.6 ng/ml; normal in summer: 10–60). She was thus diagnosed with pseudopseudohypoparathyroidism with vitamin D deficiency.
Pseudopseudohypoparathyroidism is considered to be an incomplete form of pseudohypoparathyroidism type 1a with normal blood biochemistry but abnormal morphological features of short height, excess weight, round face and brachydactyly. Her low calcium and phosphate and high parathyroid hormone levels were from vitamin D deficiency. There are reports of vitamin D deficiency mimicking pseudohypoparathyroidism type 2. Vitamin D deficiency also occurs in hyperparathyroidism but it is fairly uncommon in pseudopseudohypoparathyroidism. Thus vitamin D should always be measured in patients with abnormal calcium or phosphate level.

P105

A case of hyperaldosteronism due to bilateral macronodular adenomas with a renal mass in the left kidney
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Background
Cases of combined primary aldosteronism and preclinical Cushing's syndrome are reported extremely rare.
Highlight of a report
An unusual case of hyperaldosteronism due to bilateral macronodular adenomas with a left renal mass in a 55-year-old male is presented. MRI scan of the abdomen revealed a 4.5 cm mass of the right adrenal and two nodules in the left adrenal sized 2 and 3 cm, respectively in diameter. Additionally, a large mass on the left kidney sized 10×12 cm was also detected. Increased plasma aldosterone, decreased serum potassium and low plasma renin activity were consistent with aldosterone-producing adenoma. Although cortisol urine levels were marginally elevated, serum cortisol diurnal variation was normal, with suppression of cortisol after 1 mg of dexamethasone. Adrenal scintigraphy with ^{131}I-6-β-iodomethyl-norcholesterol, after 2 days administration of cortisol, showed an uptake of the right adrenal mass with inhibition of the contralateral adrenal gland. Kidney scintigraphy with 99MTC-DTPA showed a normal radioactive distribution with the left renal mass appearing photopenic.
Histopathology of the nodules showed clear cells admixed with paradoxical hyperplasia of zona glomerulosa and fasciculata. The left renal mass primarily composed of collagenized hyalinized fibrous tissue with many inflammatory cells surrounding necrotizing tissue with hemorrhage and cholesterol crystals. After adrenalectomy the patient's blood pressure was normalized.
Conclusion
This is the first case in the literature of bilateral involvement of the adrenals causing hyperaldosteronism with a concurrent renal mass.

P106

Coexistance of parathyroid tumors with metachronous metastasis to the thyroid from renal clear cell carcinoma 20 years after nephrectomy
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We report a rare case of a solitary metachronus metastasis of renal clear cell carcinoma (RCC) in the thyroid coexisting with parathyroid tumors. The patient had undergone 20 years ago radical left nephrectomy for RCC. Upon presentation, the initial diagnosis based on clinical and echo findings was multinodular goiter. Fine needle aspiration (FNA) cytology of a growing non-functioning nodule of the right thyroid lobe revealed clear cells with oxyphilic granules suggesting RCC. Immunohistochemical studies showed the tumor cells to be negative for thyroglobulin staining. In addition immunostaining with keratin ae1–ae3, epithelial membrane antigen, and vimentin gave positive results, confirming further the diagnosis of metastatic RCC in our case. The coexisting parathyroid tumors were assessed preoperatively by Tc-99m substraction

scintigraphy, intraoperatively by monitoring parathyroid hormone and post-operatively by histology.

P107

Adrenal incidentalomas: which do we send for operation?

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Due to the increased availability and use of radiology, endocrinologists are referred patients with incidentally discovered, clinically silent adrenal masses with increased frequency. Controversies persist regarding their investigation and management. We present two cases focusing in particular on the role of radiology in the management algorithm.

A 43-year-old male underwent a CT urogram, which revealed an incidental 3×3×2 cm right adrenal mass. The patient did not have symptoms suggestive of mineralo- or glucocorticoid excess, but had been noted to have borderline hypertension. MRI scan confirmed a mass with high signal intensity on T2. Initial and repeated biochemistry over 18 months showed normal urinary metanephrines, negative investigations for Cushing's syndrome and normal PRA/Aldosterone ratio. In May 2007, 2 years after his initial presentation, routine urine tests revealed elevated noradrenaline and normetadrenaline, in keeping with a diagnosis of phaeochromocytoma. MIBG confirmed increased activity within the right adrenal mass. This case illustrates the importance of continued biochemical testing for phaeochromocytoma when the radiology is suggestive.

A 71-year-old female was found to have a 4.5 cm right adrenal mass on abdominal CT. She had normal adrenal biochemistry on several occasions. Physical examination was unremarkable and her blood pressure was 140/80 mmHg. Based on the size of the tumour she underwent laparoscopic adrenalectomy. Histology showed a haemangioma of the adrenal medulla. There was no evidence of malignancy or an adrenal medullary or cortical endocrine cell tumour.

Careful review of abdominal radiology is important when investigating adrenal masses and may obviate the need for surgery in some patients, while prompting continued biochemical evaluation for endocrine disease in others. We propose that a greater emphasis should be placed on radiological review in current algorithms, but recognise that discharge or ongoing follow-up of hormonally silent radiologically stable lesions is still a dilemma.

P108

Prolonged QT interval on the electrocardiogram in hypogonadal men

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Background

QT interval reflects cardiac ventricular repolarization and, if prolonged, increases the risk of malignant arrhythmias such as torsade de pointes. QT interval duration is similar in boys and girls during childhood but shortens in males after puberty. In fact, reference limits for QTc in adults reflect this difference, i.e. lower than 460 ms in women and lower than 440 ms in men. Experimental studies suggest that testosterone is the major contributor to shortening of QT interval in men. Purpose of this study is to evaluate the length of ventricular repolarization in male hypogonadal patients.

Methods

The study was performed in 32 patients with secondary (25 patients) and primary hypogonadism (7 patients with Klinefelter's syndrome) and 32 age-matched eugonadal men. Patients were tested in basal conditions and after adequate testosterone replacement therapy. Twelve-lead ECG recordings were obtained and QT intervals corrected for heart rate according to Bazzett's formula, i.e. $QTc = QT/\sqrt{RR}$ interval.

Results

Prevalence of abnormal QTc was significantly higher in hypogonadal patients (16% vs 0% in controls, $P<0.05$). QTc interval normalized on hormone replacement therapy in patients presenting prolonged measurements in the hypogonadal state. Heart rate and left ventricular mass did not differ among the two groups and no known QT-prolonging factor was evidenced in patients with abnormal QTc values.

Conclusions

Testosterone deprivation leads to an increased prevalence of prolonged QTc interval and hypogonadal men appear at higher risk for cardiac arrhythmias. This study reveals an additional feature of male hypogonadism which should be corrected by testosterone replacement therapy.

P109

Recurrent silent and post-partum thyroiditis in a single patient: evidence for a common aetiology

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Silent and post-partum thyroiditis are autoimmune conditions, which result in a triphasic thyroid hormone disturbance. They are distinguished by the later condition's relation to pregnancy. Their association in the same patient resulting in recurrent episodes of silent thyroiditis suggests a common aetiology.

We report a 32-year-old female with post-partum and recurrent silent thyroiditis continuing over a decade. She presented post-partum with thyroid overactivity, followed by hypo- and euthyroidism. Over the next 10 years, she had seven episodes of hyperthyroidism; thyroid function tests in the interim periods showed euthyroidism or compensated hypothyroidism. During one toxic episode, a raised thyroglobulin level (189.1 μg/l) was found and a thyroid pertechnetate scan showed no uptake. She never exhibited clinical evidence of Grave's disease (no goitre, infiltrative orbitopathy or dermopathy) nor of subacute thyroiditis (no viral prodrome and no neck tenderness). The occurrence of recurrent hyperthyroid episodes alongside raised thyroglobulin levels, the absence of neck tenderness and scintigraphic findings indicate she had a combination of post-partum and recurring episodes of silent thyroiditis.

Painless silent and post-partum thyroiditis are normally self-limiting and non-recurring conditions. They show a triphasic thyroid hormone response of thyroid overactivity, followed by hypothyroidism and recovery to a euthyroid state. A minority of patients remain permanently hypothyroid. Our patient showed this classic hormone profile alongside raised thyroglobulin levels (excluding thyrotoxicosis factitia) and low uptake on thyroid scintiography (excluding Graves disease). Silent thyroiditis is uncommon but may be underdiagnosed and there is limited information on recurrence rates, variably described in between 5 and 65% of patients. The association of both recurrent silent and post-partum thyroiditis in our patient suggests these are likely to be a single disorder with a common aetiology. Radioiodine ablation of the thyroid is planned to prevent relapse.

P110

Successful pregnancy outcome in a woman with acromegaly treated with lanreotide before and briefly during the first trimester of gestation

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A 39-year-old woman presented with a progressive enlargement of her feet, hands, tongue, nose and ears, headaches, excessive sweating, weakness and infertility. She was diagnosed with acromegaly at the age of 36, but had initially refused any kind of treatment. For a period of 3 years, the size of pituitary adenoma had enlarged from 10×12×20 to 13×19×22 mm, and the level of GH had increased from 62.4 to 104 mU/I. Preoperative administration of somatostatin analogues was recommended.

At the course of treatment with lanreotide she conceived and decided to preserve pregnancy. Lanreotide was discontinued after pregnancy was confirmed. In the first trimester, her GH and IGF-1 were in the normal range, followed by gradual increase since the second trimester. No complications or visual impairment during gestation were observed, and a healthy female infant (weight 3560 g, length 52 cm) was delivered at term.

After delivery high levels of GH and IGF-1 without significant enlargement of adenoma were registered. When breast feeding was stopped, lanreotide treatment was resumed with marked effect: GH and IGF-1 levels have gradually dropped from 189 to 11.9 (reference range 0.2–13.0 mU/l) and from 1388 to 388 (reference range 101–267 ng/ml) respectively. The patient continues to receive injections of lanreotide. The baby remains developmentally normal for 8 months of the follow-up.

There are few reported uncomplicated pregnancies in acromegalic women, having received depot somatostatin analogues at different stages of gestation period. We present a case of conception and successful pregnancy outcome in a 39-year-old woman with somatotropinoma, treated with lanreotide before and briefly during the first trimester of gestation.

P111

Androgenic alopecia and hirsutism in a 73-year-old woman: careful re-evaluation of 'normal' imaging findings may lead to a rare diagnosis

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A 73-year-old woman developed androgenic alopecia and progressive hair growth on the chest, back and abdomen over the course of 3–4 years. Menarche was at age 16. She had irregular menstrual periods subsequently but gave birth to three children. She reached menopause at age 40. No history of weight loss or sweating was reported. At presentation, we saw a 73-year-old lady with pronounced hirsutism (Ferriman–Gallway-score 24/36). No virilizing signs of the external genitalia were present but there was impressive androgenic alopecia. BMI was 31.5 kg/m^2, blood pressure was 160/85 mmHg, pulse was 68 bpm and regular. The abdomen was soft; there were no masses palpable except for an incisional hernia. Hormonal evaluation of hyperandrogenism revealed elevation of total testosterone level to the neoplastic range. DHEA-S, androstendione, 17-OH-progesterone and a 24 h urine collection for cortisol were normal. Transvaginal ultrasound of the ovaries was 'normal' and abdomino-pelvic CT-scan showed no tumorous lesions of the adrenal glands and ovaries. Careful re-evaluation of the CT-scan indicated that the ovaries appeared to be too large in relation to age. We postulated excess ovarian production of testosterone and performed bilateral ovarectomy. The histopathological examination of the ovaries showed stromal hyperthecosis. The postoperative testosterone-level normalized, hirsutism and androgenic alopecia improved on follow-up.
Ovarian hyperthecosis with hyperandrogenism is a rare cause of hirsutism and virilization in postmenopausal women and is difficult to diagnose as it may elude imaging studies. It should be included in the differential diagnosis in postmenopausal women with recent-onset of androgen excess. Careful intraoperative examination of even normal-appearing ovaries is imperative, particularly if no other cause of marked androgen excess is found.

P112

Children with growth retardation due to Rathke cleft cyst

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Rathke cleft cysts (RCCs) are non-neoplastic sellar lesions derived from remnants of Rathke's pouch, and mostly asymptomatic. Symptomatic RCCs occur usually in middle-age, are >1 cm, and cause pituitary hypofunction, diabetes insipidus, hyperprolactinemia or visual impairment. In children, RCCs are rare and usually asymptomatic. However, symptomatic cases may present with growth retardation and diabetes insipidus. We report two children with symptomatic RCC manifesting as growth retardation.
First case
A 14-year-old boy was admitted because of growth retardation (S.D.S. height -2.5) and delayed puberty. Serum levels of SmC (21 ng/ml), cortisol (4.23 μg/dl), fT4 (0.61 ng/dl) and T3 (0.8 ng/ml) were low, TSH (1.84 μIU/ml) was normal and PRL (2556 μIU/ml) elevated. Stimulation tests of growth hormone (GH) secretion showed low GH (GHmax 1.6 ng/ml). Pituitary MRI revealed an intra- and suprasellar mass $1.2\times3.8\times2.3$ cm with solid and cystic elements. Visual fields were normal. After the start of cortisone and thyroxine the child presented polydipsia, and polyuria. Diabetes insipidus was diagnosed with water deprivation test and treatment with desmopressine was started. Trans-sphenoidal dissection was performed. Histological examination showed a RCC. Postoperatively, treatment with recombinant human growth hormone (rhGH) was started improving child's height.
Second case
A 12-year-old girl was admitted because of growth retardation (S.D.S. height -2.5). Hormonal tests were normal except for low level of SmC (220 ng/ml) and stimulation tests of GH secretion (GHmax 4.2 ng/ml). Pituitary MRI revealed an intrasellar cyst $1.5\times1.2\times1.2$ cm. Visual fields were normal. Trans-sphenoidal dissection was performed. Histological examination showed a RCC. Post-operatively, the child presented polydipsia, polyuria and a water deprivation test revealed diabetes insipidus and she started treatment with desmopressine. Height was improved with rhGH.
Conclusion
RCC should be considered in growth retardation and/or hypopituitarism in children. Pituitary MRI can reveal the lesion. Early detection and treatment is mandatory to prevent or to inverse growth retardation and other pituitary hormones failure.

P113

Unresectable huge sternal and mediastinal metastasis of follicular thyroid carcinoma; radiotherapy as first-line and palliative therapy

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Distant metastases as initial manifestation of follicular thyroid carcinoma is rare. We report a case of an unusual initial presentation of follicular thyroid carcinoma on follow-up. A 52-year-old woman presented with a 12-month history of progressively enlarging mass in the anterior chest wall. The mass was fixed to the chest wall, measuring 12×10 cm in diameter. Computed tomography demonstrated a lobulated soft-tissue mass ($17\times11\times6$ cm) destructing sternum and extending into the anterior mediastinum. There was no lung metastasis. Invasion of tumor to the ascending aorta, superior vena cava, and right atrium could not be excluded. Multiple lymph nodes were observed in the supraclavicular regions. Ultrasonography of the thyroid gland showed 46×37 mm nodule in the left lobe with milimetric gross calcifications and cystic-necrotic areas. Hyperthyroidism was detected. Biopsy from this nodule and the sternal mass revealed typical histology of follicular carcinoma. She was considered inoperable. Since there was huge tumor burden and iodinated contrast exposure for several times during evaluation, we decided to treat the patient with external beam radiotherapy (EBRT) rather than radioiodine as first-line therapy. After a course of conventional radiation with 50 Gy in 25 fractions over 4 weeks, encompassing the thyroid bed and the gross disease, tumor regressed remarkably in 6 months. In conclusion, when surgical resection is not possible, EBRT may be used for palliative purpose to obtain local control for extensive disease as first-line therapy. The indications of EBRT for differentiated thyroid cancer still remain poorly defined.

P114

Severe secondary osteoporosis in a patient with systemic mastocytosis stabilized by therapy with low-dose pegylated interferon alpha

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Objectives
We report on a case of a 32-year-old patient with urticaria pigmentosa, the cutaneous form of mastocytosis, since the age of 13. A bone marrow biopsy revealed systemic mastocytosis disease. In 2005, after a minor trauma, the patient had an X-ray of the spine which showed a new fracture of the thoracic vertebrae 3–4. Further DXA examination of the bone mineral density showed osteoporosis (*T*-Score -2.8 lumbar vertebrae; right/left femur $-1.0/-0.9$). As interferon alpha is effective in mastocytosis and the pegylated form of the drug is better tolerable, the clinical response of the pegylated interferon alpha was analyzed.
Methods
A treatment with pegylated interferon alpha 2a 90 μg s.c. once a week for 6 months was initiated.
Results
No further fractures occurred and the bone mineral density increased (after 6 months *T*-Score 2.2 lumbar vertebrae; right/left femur $-0.9/-0.7$). Side effects of the treatment were minor compared to the benefit.
Conclusion
Low-dose pegylated interferon alpha therapy for 6 months in a young man with severe secondary osteoporosis due to systemic mastocytosis improved the bone mass density as well as clinical parameters. The drug appears effective in treating mastocytosis with osteoporosis and should be examined in more patients.

P115

The influence of application site on testosterone serum concentrations after transdermal testosterone gel

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Background
Testosterone (T) in a hydroalcoholic gel is commonly prescribed as androgen replacement therapy and may not be accompanied by peaks and troughs of serum T levels as seen in T-injectables. We assessed in 17 subjects the differences in serum testosterone (sT) and free T (fT) levels during four different application periods.
Methods
Patients where on daily 50 mg Androgel®, for 7 consecutive days in each cycle, on day 8 serum was sampled from both antecubital veins at 9.00 pm. Period 1 started with application at both shoulders and right upper arm up to 10 cm from the elbow pit. Period 2 was equivalent to period 1 except that T-gel was applied to the left arm. Period 3 was similar to the second except T-gel was applied up to the left elbow pit. Period 4, T-gel was solely applied on the abdomen.
Results
No significant differences in (free) T levels were observed between regimens 1 and 2. However, in period 3 sT and fT levels (median$\pm$S.D.) sampled from the left arm were higher compared to the right arm (sT left 15.60 ± 22.17 nmol/l, right 12.20 ± 4.70 nmol/l, $P=0.011$; fT left 0.39 ± 0.74 nmol/l, right 0.29 ± 0.15 nmol/l $P=0.012$). Significant lower sT (7.35 ± 3.45 nmol/l, $P<0.05$) and fT (0.16 ± 0.08 nmol/l, $P<0.05$) were observed when T-gel was applied to the abdomen compared to all other applications.
Conclusion
Peaks of sT concentrations are observed during T-gel therapy with different application regimens. Serum sampling within the T application area may reveal high T levels, possibly due to local T-accumulation. This should be taken into account with interpretation of sT levels and dose titration during transdermal androgen substitution. Efficacy of T application at the shoulders may be higher compared to application at the abdomen.

P116

Increased frequency of Cushing's disease in patients with polycystic ovary syndrome

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Background
Cushing's disease is defined as ACTH dependent pituitary Cushing's syndrome and is rare with an estimated incidence of 5–25 per million/year. Polycystic ovary syndrome (PCOS) is a heterogenous disorder affecting up to 10% of women in the reproductive age and shows clinical overlap with Cushing's syndrome (obesity, hirsutism, cycle abnormalities, etc). An increased frequency of Cushing's syndrome in selected patient groups has been postulated previously but is unknown in patients with PCOS.
Patients
Three hundred and seventy-eight patients with PCOS according to the Rotterdam criteria were included in the study. All patients received a clinical and laboratory evaluation including ACTH and basal cortisol levels. In case of abnormal parameters, further investigations were performed including Dexamethason suppression test, 24 h urine secretion of cortisol, midnight salivary cortisol levels and imaging studies.
Methods
Clinical study, biochemical, functional and imaging studies.
Results
In 4 out of 378 patients, autonomous overproduction of cortisol was detected. Subsequent investigations revealed a pituitary tumor in all four of these patients. The affected patients underwent hypophyseal surgery with curative effect on the hypercortisolemia and improvement of associated hormonal and metabolic disturbances.
Conclusions
A frequency of 4/378 (1.05%) for Cushing's disease in the PCOS patient group is much higher than the expected frequency in the general population. We propose that Cushing's syndrome is much more common in PCOS than previously supposed. The current criteria for PCOS establish the diagnosis only if other endocrine disorders have been excluded. This implies careful exclusion of autonomous cortisol overproduction in all patients with PCOS in order to detect a potentially curable underlying disease.

P117

Simultaneous detection of a heterozygous deletion in the STX16 gene and loss of methylation at GNAS1A by methylation-specific MLPA in two patients with pseudohypoparathyroidism type 1b (PHP 1b)

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The GNAS locus (chromosome 20q13) yields multiple transcripts, including the stimulatory G protein subunit α (Gsα), NESP55, GsαXL and two noncoding RNAs, the GNAS1A-transcript (A/B) and the antisense transcript (AS). The corresponding promoters show a complex methylation pattern resulting in an allele-specific imprinting, with maternal expression of NESP55 and paternal expression of GNAS1A, GsαXL and AS. Gsα in most tissues is derived from both alleles, except for the renal proximal tubules where it is exclusively maternally expressed. Expression of Gsα from the maternal allele in the kidney apparently requires silencing of GNAS1A by methylation, loss of methylation at GNAS1A leads to suppression of Gsα. Different endocrine disorders are associated with inactivating or activating mutations as well as impaired imprinting at the GNAS locus. Impaired imprinting of GNAS1A on the maternal allele has been identified as the underlying cause of PHP1b, a disease characterized by hypocalcemia, hyperphosphatemia and elevated serum levels of parathyroid hormone (PTH), due to renal resistance to PTH. We here describe two patients, sisters aged 30 and 33 years, respectively, who presented with hypocalcemia (1.69 and 1.7 mmol/l; normal range 2.2–2.65 mmol/l), hyperphosphatemia (1.54 and 1.68 mmol/l; normal range 0.87–1.45 mmol/l), and elevated PTH (378 pg/ml; normal range 10–65 pg/ml) but no other endocrine abnormalities. Sequencing of the Gsα gene did not reveal any mutations. By methylation-specific MLPA we detected a heterozygous deletion of exon 5 and 6 of the syntaxin-16 (STX16) gene and a loss of methylation at GNAS1A. A deletion within STX16, located about 220 kb upstream of GNAS1A, resulting in loss of methylation at GNAS1A, has been reported in multiple kindreds with PHP1b. Methylation-specific MLPA offers the possibility of detecting deletions as well as impaired methylation at the GNAS locus in a single assay.

P118

TSH-secreting pituitary adenoma with GH-secretion

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Hyperthyroidism due to TSH-secreting pituitary adenoma is a rare disease and accounts for 0.4% of all pituitary tumors. About 15% of these tumors show co-secretion of prolactin or growth hormone.
We report the case of a 44-year-old man, presenting with sudden onset of tremor and agitation and central thyrotoxicosis (TSH 1.01 µU/ml, fT3 8.44 pmol/l, fT4 30.2 pmol/l). Ultrasound of the thyroid gland and TSH-receptor antibodies were normal. Imaging of the pituitary demonstrated a 12 mm pituitary macroadenoma. Evaluation of pituitary function revealed no signs of hypopituitarism, but an elevated IGF-1 of 397 ng/ml and growth hormone (GH) of 2.8 ng/ml without any clinical signs of acromegaly. GH was suppressed in an oral glucose tolerance test to a nadir of 0.6 ng/ml. The macroadenoma was completely removed by an endoscopic transsphenoidal approach. Histology showed a pituitary adenoma (WHO grade I) with marked expression of GH ($>30\%$), as well as TSH and α-subunit. Three months after surgery, the patient was completely asymptomatic with normal thyroid function (TSH 0.73 µU/ml, fT3 5.17 pmol/l, fT4 18.7 pmol/l) and normal IGF-1 (132 ng/ml) and GH (0.1 ng/ml). There were no signs of hypopituitarism and imaging of the brain showed no residual tumor. The lack of acromegalic features is probably due to the sudden onset of hyperthyroidism and only mildly elevated GH and IGF-1.
This case highlights the potency of plurihormonal secretion of pituitary adenomas. Transsphenoidal resection is the therapy of choice for TSH-secreting tumors with reported cure rates of 50–80%.

P119

Atypical laminopathy revealed by non-insulin dependent diabetes and conduction disturbances

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LMNA mutations cause a wide range of diseases involving either specific tissues in isolated fashion (cardiac and skeletal muscles, nerve, adipose tissue) or several tissues in a generalized way (premature ageing syndromes…). The cardiac disease is defined by conduction and rhythm disturbances, followed by dilated cardiomyopathy, heart failure, and sudden cardiac death. We report an unusual case of laminopathy revealed by diabetes associated with conduction disturbances related to a not-yet described *LMNA* mutation. A 36-year old woman was admitted because of hyperglycemia (11 mmol/l) discovered after a 12-kg weight loss. Clinical examination showed a BMI that was still 34 with android repartition, waist acanthosis nigricans, hyperlordosis and short hands. Blood pressure was 130/67 mmHg and heart frequency 40 bpm related to an asymptomatic auriculo-ventricular conduction block requiring pace maker implantation. HbA1c level was 9.4% with mild increase of liver enzymes (ALAT: 64, ASAT 85 UI/l ($N<30$), cholesterol (2.31 g/l; $N<2.0$) and triglycerides (2.49 g/l; $N<1.5$). Blood magnesium level was 15 mg/l (N: 18–22). A heterozygous deletion of C 2159 in exon 5 of the *LMNA* was identified in the propositus but not in eight family members. Nevertheless, family inquiry had shown a history of dyslipidemia and premature sudden death (<45 years old) in the mother who also had a pace-maker, grand-mother who had 'muscular weakness', and two brothers of this grand-mother. The son of one of them also had a pace-maker since the age of 35. To conclude, this case report shows that cardiac laminopathy may be revealed by diabetes and atypical lipodystrophy, linked to new *LMNA* mutation. However, suggestive phenotypes in the other family members with absence of the described pathogenic genotype, rise the question of the association with another gene mutation or a role of hypomagnesemia.

P120

A Korean adult-onset type II citrullinemia with confirmed SLC25A13 mutation: a case report

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Adult-onset type II citrul this rare metabolic disease for the adult patient who exhibits mental changes and hyperammonemia without liver or brain diseases. Recently, the *SLC25A13* gene, which encodes the mitochondrial aspartate glutamate carrier protein named citrin, is demonstrated to be responsible for adult-onset type II citrullinemia. While there have been multiple cases reported in the Japanese population, there is no report of adult-onset type II citrullinemia with confirmed SLC25A13 linemia is an autosomal recessive disorder of the amino acid metabolism caused by a deficiency of liver-specific argininosuccinate synthetase activity. This disease may occur at any stage in life with recurrent episodes consisting of neurological signs and symptoms such as disorientation, abnormal behavior (aggression, irritability, and hyperactivity), seizure, coma, and potential death from brain edema, which result from hyperammonemia. It should be considered that mutation in Korea. However, we experienced a 39-year-old female who suffered from generalized weakness, dizziness, and lethargy and was diagnosed with adult-onset type II citrullinemia based on highly elevated plasma citrulline and ammonia and the *SLC25A13* gene mutation.
Thus, it is reported the first case of adult-onset type II citrullulinemia with confirmed SLC25A13 mutation in Korea with a brief review of the related literature.

P121

Primary squamous cell carcinoma of the thyroid

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Background
Primary squamous cell carcinomas of the thyroid are uncommon entities with less than 100 cases described in the literature.
Case report
We report on the imaging findings in a rare case of primary squamous cell carcinoma of the thyroid that was found to be the primary tumour of humerus and cervical lymph node metastases. Other sources of squamous cell cancer were ruled out by a careful search, including whole body computed tomography (CT), positron emission tomography/CT after intravenous F-18 fluorodeoxyglucose, complete otorhinolaryngological examination, and panendoscopy.
Conclusion
Primary squamous cell carcinomas of the thyroid, though very rare, should be included in the differential diagnosis of metastatic disease of squamous cell cancer.

P122

Cardiometabolic risk factors in type 2 diabetic patients according to the definition for metabolic syndrome of International Diabetes Federation

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Objective
The study was to characterize metabolic syndrome (MS) in type 2 diabetic patients (T2D pts) according to the definition of International Diabetes Federation (IDF) and to test the hypothesis that this definition identifies insulin resistant subjects.
Materials and methods
Three hundred and eighty-three (194 females, 189 males) T2D pts of age 62.2±10.4 years, HbA1c 7.5±1.4%, BMI 30.8±4.8 kg/m^2 (mean±S.D.) took part in the study. Fifty persons with normal glucose tolerance (NGT) of mean age 60.7±11.2 years served as a control group with regard to insulin sensitivity (IS). It was determined as a glucose metabolized (M; mg/kg per min) using a manual hyperinsulinaemic euglycaemic clamp technique and homeostasis model assessment of insulin resistance (HOMA-IR). The study was approved by Local Ethical Committee.
Results
According to the definition of IDF, MS was diagnosed in 76.5% of the T2D pts (82% females, 70.9% males). The highest percent of the MS pts (75.1%) were characterized by raised blood pressure, followed by those with reduced HDL cholesterol (63.5%), raised triglycerides (72.3%) and combined dyslipidaemia was established in 42.3% of the MS pts. IS of the MS pts M 3.238±1.673 was significantly lower compared to that of the pts with no MS (non-MS pts) M 6.893±3.846 and control group M 6.296±3.176 mg/kg per min, both $P<0.001$. HOMA-IR of the MS pts 6.02±1.69 was significantly higher compared to that of non-MS pts 3.07±1.27 and control group 3.54±1.92, both $P<0.001$.
Conclusions
The diagnostic criteria for metabolic syndrome of IDF definition are easily applicable in routine clinical practice that makes it a very useful tool for early treatment of cardiometabolic risk factors. Nevertheless that insulin resistance is not included in IDF definition, according to our data, it identifies type 2 diabetic patients with insulin resistance, as well.

P123

Fungal thyroiditis

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Case
A 38-year-old HIV positive lady presented with a neck swelling and neutropenic sepsis. Examination of the neck revealed a firm diffuse non-tender goitre with

cervical lymphadenopathy. She also had generalised lymphadenopathy, splenomegaly and miliary opacities in the lungs on the chest radiograph. Disseminated cryptococcal infection was diagnosed following isolation of the organism in blood and bone marrow. A radioisotope scan of the thyroid showed no uptake in the right lobe and reduced uptake in the left. Anti-thyroid microsomal antibodies were negative. She had hypothyroidism with a free T4 of 5.3 pmol/l (NR 11.5–23.2) and TSH 46 mU/l (NR 0.3–3.5), and was given thyroxine replacement. Histology from a core biopsy of the thyroid gland showed extensive replacement of thyroid tissue with organisms with a cell size considerably larger than an erythrocyte, surrounded by a halo effect. Cryptococcus was confirmed by Grocott silver stain.

Discussion

Both goitre and thyroiditis due to opportunistic infection have been described in patients with HIV infection. Cryptococcal infection presenting as hypothyroidism associated with goitre in a patient with HIV could be misdiagnosed as Hashimoto's thyroiditis if infectious aetiologies are not considered. Thyroiditis in HIV may be due to suppurative thyroiditis (streptococcal or staphylococcal), Tuberculous thyroiditis, Pneumocystis carinii or Cryptococcal infection. A lymphocytic auto-immune thyroiditis has also been reported as a specific AIDS-related lesion. Thyroid function tests may be misleading in HIV because of rising thyroxine binding globulin, effects on the pituitary–thyroid axis due to hypermetabolism, and a sick euthyroid syndrome in the late stages of the disease, although free T3 concentrations are relatively well maintained in the early stages. We conclude that thyroid FNA and in some cases, core biopsy will have to be carried out in HIV patients with goitre and hypothyroidism to obtain an accurate diagnosis.

P124

Increased levels of thyroid hormone and a non-suppressed TSH in two patients due to different mutations in T3 receptor beta (TRβ)

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Introduction

Resistance to thyroid hormone (TH) can be caused by a failure of TH to enter the cell, or by a lack of action of intracellular TH.

Cases

Patient A, a 60-year-old man, presented with an elevated TSH (15.8 mU/l (reference range: 0.4–4.3 mU/l)), an elevated FT4 (29.5 pmol/l (11–25 pmol/l)), and a normal T3. Reverse T3 (rT3), the inactive metabolite of T4, was clearly elevated (0.82 nmol/l (0.14–0.34 nmol/l)). His medical history revealed a partial thyroidectomy because of hyperthyroidism. After disease recurrence, he was treated with radioactive iodine in 2001. At presentation, he used 75 μg of thyroxine daily. Additional evaluation showed no signs of TH dysfunction, and other hormonal axes functioned normally. The differential diagnosis consisted of resistance to TH, a TSH producing tumor, or an altered TH transporter or metabolizing enzyme. We identified a novel mutation in TRβ (Leu456Phe), which was not found in his family members. The effect of the Leu456Phe mutation is currently being tested.

Patient B, a 33-year-old man, presented with an elevated FT4 (42.1 pmol/l), an elevated T3 (3.16 nmol/l (1.4–2.5 nmol/l)), and an elevated rT3 (0.72 nmol/l). Nevertheless, he had a non-suppressed TSH (3.31 mU/l) and no symptoms of hyperthyroidism. Additional laboratory testing showed normal function of other hormonal axes, and a normal TRH-test. His mother and aunt had elevated levels of FT4 and a non-suppressed TSH as well, and one of them had an increased metabolic rate. Genetic testing revealed a mutation in TRβ (Glu462Lys) in all three subjects. This mutation was previously described in three unrelated families, resulting in a similar phenotype.

Conclusion

We identified two different mutations in TRβ, resulting in TH resistance in four subjects. High levels of TH, a normal or slightly elevated TSH, and usually mild symptoms are suggestive for the diagnosis.

P125

Effects of gender on clinical and biochemical features in Cushing's disease

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Cushing's disease (CD) occur more frequently in females than in males, but in men this disease seems appear at a younger age and with more severe symptoms and clinical course. Aim of this study was to compare clinical features and biochemical indices of hypercortisolism in male and female patients with CD.

Methods

Forty-five patients (35 females and 10 males) with confirmed CD were analyzed. All patients underwent a complete biochemical and endocrine assessment; in selected case bilateral inferior petrosal sinus sampling (BIPPS) was also carried out.

Results

At diagnosis, males were younger than females (mean age was respectively 33.6 ± 12.8 years vs 42.9 ± 13.4 years). Males presented more severe hypercortisolism with significantly higher ACTH levels, UFC, morning plasma cortisol, midnight plasma cortisol, cortisol after 1 mg dexamethasone suppression test (DST) and cortisol after high-dose DST. No significant differences have been observed between males and females regarding sensitivity and specificity of DST (low and high dose), ACTH and cortisol responses to CRH and DDAVP test and basal and post-CRH ACTH gradient at BIPPS. MRI detected a pituitary adenoma in 88% of patients with a significant prevalence of macroadenomas in males (40% vs 6%). In the other patients the MRI showed no evidence of tumor with a slight prevalence in man. Males patients presented high blood pressure values with higher prevalence of hypertension. The prevalence of osteoporosis and symptomatic fractures were higher in males patients compared with females. Conversely, no differences between sexes were found in BMI, fasting glucose, cholesterol and triglycerides.

Conclusion

Our study confirms that male patients present a CD at younger age with a more florid clinical presentation and a severe hypercortisolism; this finding might be at least due to a more prevalence of ACTH-secreting macroadenoma in these patients.

P126

Failure of restrictive bariatric surgery – sleeve gastrectomy – in severe hypothalamic obesity secondary to Langerhans cell histiocytosis: a case report

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Langerhans cell histiocytosis (LCH) is a rare disease often involving the hypothalamo-pituitary axis. Extreme obesity is frequent, but effective treatment is not. Malabsorptive bariatric surgery techniques are effective for weight loss in hypothalamic dysfunction. Laparoscopic sleeve gastrectomy (LSG) has recently emerged as a restrictive bariatric procedure to be used before biliopancreatic diversion or gastric bypass, thus reducing surgery-associated morbidity in particularly obese patients.

Report

We report a 20-year-old man, current smoker, puberty at thirteen, diagnosed as having LCH involving lungs and maxilla treated with butazolidin. He related polyuria, polydipsia and hyperphagia. Normal libido, no erectile dysfunction. Weight 198 kg, height 190 cm, BMI 54.84 kg/m^2. Scarce body hair, goiter, bilateral gynecomastia. Testis 3–4 ml. Obstructive sleep apnea.

Laboratory

pOsmolarity 289 mosm/kg (270–290), Uosmolarity 79 mosm/l per kg. TSH 13 mcU/ml (0.3–5), T4L 0.9 ng/dl (0.8–1.7), positive anti-TG and anti-TPO AB. Undetectable GH, LH and FSH, f-testosterone 1.1 pg/ml (9–55), IGF-I 214 ng/ml, PRL 329 mcUI/ml (79–208). Normal ACTH and ACTH-stimulated cortisol.

NMR

Infiltration of hypothalamus and pituitary stalk.

Diagnosis

Central diabetes insipidus, primary autoimmune hypothyroidism, secondary hypogonadism, morbid obesity.

Treatment

Desmopressin, levothyroxine, testosterone gel. Hypocaloric diet, exercise, behaviour therapy, and sibutramine were of no benefit. Weight reached 220 kg (BMI 60.9 kg/m^2). A LSG was used as first-step intervention. Maximum weight loss was 37 kg (EWL% – excess weight loss percentage – 26.4%) four months following surgery. Due to voracious eating the patient reached 198.8 kg in the following 6 months.

Conclusion

Restrictive bariatric surgery, such as LSG, does not seem to be effective as a first step prior to a malabsorptive procedure in especially obese subjects with hypothalamic dysfunction.

P127

Is metoclopramide test useful in differentiation of the clinical form of hyperprolactinemia?

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Metoclopramide (MCP) is a selective blocker of dopamine D2 receptor, responsible for the dopamine inhibition of prolactin (PRL) secretion from the pituitary. In normal conditions, administration of the proper dose of MCP leads to increase of PRL concentration in the blood serum. This response was applied in the diagnostic test (MCP test), useful to differentiate between hyperprolactinaemia linked with organic causes (prolactinoma and other lesions of hypothalamo-pituitary region) and so-called functional hyperprolactinaemia. However, recently the value of this test is a subject of controversy. Because of that we evaluated the usefulness of MCP test in our clinical material. We analyzed 228 patients with hyperprolactinaemia, hospitalized in the Department of Clinical Endocrinology, Medical University of Lodz, in 2006. In all patients, MCP test and magnetic resonance imaging (MRI) were performed. In 48 (21%) patients, the pathological changes in MRI (pituitary macro- and microdenomas, pituitary stalk suppression) were found and these patients were included to the organic hyperprolactinaemia group. The remaining 180 (78.9%) patients without alterations in MRI were included to the group of functional hyperprolactinaemia. The administration of MCP in patients with pituitary tumors and with stalk suppression induced the slight increases of PRL concentration in 60 min of the test (30 and 40%, respectively). In patients with functional hyperprolactinaemia, a sharp increase of PRL concentration (usually over 300%) was found. These results indicate that the MCP test possesses the diagnostic value and may be usefull especially in cases with marked basal levels of PRL.

P128

Conversion from hypothyroidism due to Hashimoto's thyroiditis into Graves' hyperthyroidism in a case of thyroid hemiagenesis

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Background
Thyroid hemiagenesis (TH) is a rare inborn anomaly, occurring if one of the thyroid lobes fails to develop. Due to mostly asymptomatic course, it is usually detected incidentally, during investigation of concomitant abnormalities of the thyroid function or structure.
Case report
A case of Graves' hyperthyroidism following primary hypothyroidism due to Hashimoto's thyroiditis (HT), accompanying a TH, is reported. The patient first presented symptoms of hypothyroidism at the age of 49, when HT and left thyroid lobe agenesis was diagnosed. L-Thyroxine replacement therapy restored euthyroidism. Two years later, first clinical symptoms of hyperthyroidism appeared, persisting despite the cessation of L-thyroxine and treatment with propranolol. Due to intensifying clinical and laboratory signs of thyrotoxicosis associated with an increase in thyrotropin receptor antibody concentration (TRAb), Graves' disease was diagnosed. The antithyroid pharmacotherapy by thiamazole was used. However, because of severe side effects it was discontinued, and the patient underwent radioiodine treatment. The administered therapy resulted in subsidence of thyrotoxicosis. Though, four months after ^{131}I administration, the patient developed symptoms of hypothyroidism with a marked increase in thyrotropin level, so substitution with L-thyroxine was reintroduced. Hormonal balance was soon achieved, which was reflected in the improvement of the patient's clinical state. No relapse of hyperthyroidism was detected, and a slow, gradual decrease in the TRAb level was observed. The patient, whose observation period has now reached five years, continues to be followed-up in the outpatient clinic and under L-thyroxine replacement therapy, remains both clinically and biochemically euthyroid.
Conclusions
To our knowledge, this is the second case of conversion from primary hypothyroidism due to HT into Graves' hyperthyroidism coupled with TH, reported in the literature. Each of these three entities, may influence the thyroid function in a different way, hence, systematic follow-up and individual therapeutic management is required.

P129

Ovarian hyperthecosis in a postmenopausal woman

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Ovarian hyperthecosis is a rare cause of androgenic alopecia in postmenopausal women. The physiological levels of androgens, secreted by ovarian stromal cells, are greatly increased with hyperplastic or neoplastic transformation leading to possible clinical consequences.
We report a case of 56-year-old woman with type 2 diabetes presenting with hirsuitism and a history of male pattern hair loss over a two-year period. Biochemistry showed elevated levels of testosterone 9.7 nmol/l (range: 0.2–2.9 nmol/l), extracted testosterone 8.5 nmol/l (range: 0.5–2.5 nmol/l) and androstenedione 13.1 nmol/l (range: 1.7–12.9 nmol/l) whilst gonadotropins, oestradiol, cortisol, prolactin and thyroid hormones were within the appropriate reference range. These hormone levels together with a non-suppression of testosterone during low dose dexamethasone suppression test raised the possibility that the elevated testosterone was of ovarian origin. A transvaginal ultrasound revealed bilateral solid adnexal masses, likely to be ovarian in nature, although no normal ovarian tissue was identified.
Based on the above investigations a working diagnosis of 'hyperandrogenism of ovarian origin' was made at multidisciplinary team meeting and after discussing the treatment options with the patient a bilateral oophorectomy was carried out. The left ovary measured $50\times30\times25$ mm and the right ovary $60\times30\times20$ mm and the resulting histology revealed significant bilateral ovarian hyperthecosis. In our patient, surgical therapy had an excellent result. An alternative treatment option for ovarian hyperthecosis, as reported in various case reports, is GnRH agonist therapy.
A month following surgery the testosterone levels were normalized (between 0.2 and 0.4 nmol/l) and the patient reported a clear regression of both hirsuitism and hair loss. However, surprisingly her glycaemic control deteriorated significantly after surgery (pre-surgery HbA1c: 7.4%, post-surgery HbA1c: 9.5%) and this was contrary to expectations as studies suggested that the patient could have anticipated an improvement. At present, we can offer no satisfactory explanation of this last observation.

P130

A fetus affected with a complete androgen insensitivity syndrome due to a novel mutation of AR and persistent Mullerian structures

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Complete androgen insensitivity syndrome (CAIS) is a rare X-linked disorder caused by androgen receptor gene (AR) mutations that result in complete impairment of genital virilisation. Usually CAIS patients, who have normal production of AMH by Sertoli Cells, do not show Mullerian derivatives, although the persistence of Mullerian duct derivatives up to now have been described in nine cases.
Here, we report the case of a fetus aborted at 20 weeks for genital ambiguity. In particular, during autopsy, external and internal (upper third of vagina, uterus and fallopian tubes) properly developed female genitals were found. Moreover, undescended testes characterized by a large hyperplasia of Leydig cells were also identified. Interestingly, a cousin and two aunt showed a similar phenotype. The subsequent genetic analysis demonstrated a novel mutations affecting the ligand binding domain of AR (D768V). The immunoistochemistry of the testes for AMH was positive. In previous cases described in the literature no mutations of AMH and AMHR have been described and all the hypothesis that have been suggested to explain this phenotype such as inappropriate synthesis or action of AMH, *in utero* exposure to diethylstilboestrol, impaired uptake of AR complex at nuclear level and early testicular descent so that Mullerian structures are beyond the reach of AMH action, have been eventually ruled out. So, considering that AR mutation justify only half phenotype, that Sertoli cells during fetal life don't express AR and that AMH act within the first 8 weeks of gestation, before Leydig cells start to synthesize testosterone, we ruled out a possible connection between androgen resistance and the lack of Mullerian derivatives regression. We hypothesized the presence of an unknown gene involved in AMH processing or in downstream AMH transduction, suggesting the necessity of performing linkage analysis.

P131

A long standing lymph node metastasis from occult thyroid papillary carcinoma: 10 years evolution until positive diagnosis

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Papillary thyroid carcinoma is a slow growing tumor and is reputed to have an excellent prognosis. In 10–20% of cases, the presence of lymph node metastases led to diagnosis.
Objective
To present a patient with enlarged lateral cervical masses, present for 10 years, that proved to be lymph nodes with metastatic thyroid papillary carcinoma.
Case report
We report a case of an 26-year-old female patient with 10 years evolution of enlarged cervical masses in the absence of readily palpable thyroid nodularity. Thyroid ultrasonography disclosed tow solid nodules <0.5 ml in the left lobe. Thyroid function was normal and technetium Tc 99 m pertechnetate scan revealed no 'cold' nodules. A lymph node biopsy was required and the lesions were histologically metastasis of papillary carcinoma. The patient underwent total thyroidectomy with lymph node neck dissection and superior mediastinal lymph node dissection followed by radioactive iodine therapy. After 4 months, the wholebody scan was negative and the tyroglobuline decreased from 701 to 21.97 ng/ml.
Conclusions
In a young patient with lateral cervical masses, the diagnosis of lymph node metastasis from occult papillary carcinoma should be considered. Even the diagnosis was made after 10 years of slow evolution, the right treatment (surgery and radioiodine therapy) succeeded.

P132

FSH secreting pituitary adenoma: a case report

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Gonadotroph adenomas represent 40–50% of pituitary macroadenomas. Only a small subset of these tumors secrete sufficient hormone to elevate serum gonadotropin levels (functioning gonadotroph tumors).
We are presenting the case of a 30-year-old male patient (E.R) having a characteristic symptomatology for the pituitary macroadenoma: headaches, impaired visual acuity and reduced visual field (based on ophthalmological examination). MRI (with gadolinium-based contrast agent) showed an intrasellar mass with sphenoidal, nasal and suprasellar extension, measuring 4×5 cm. Transfrontal pituitary adenomectomy was performed. Postoperative CT scan showed no remnant tumor. The patient had a favorable clinical evolution, headaches and visual symptoms improving after surgery. Postoperatory, the patient presented polyuro–polydipsic syndrome. Endocrine studies were performed only after surgery. The clinical examination indicated normal secondary sexual characters, normal testes shape and consistency, with a testes volume over the maximum Prader level. Postoperative hormonal assays indicated: secondary hipoadrenalism, central hypothyroidism, normal PRL serum level, low testosterone, LH and GH serum levels and very high FSH serum level. The substitutive hormonal therapy was initiated with: cortisol, antidiuretic hormone and thyroid hormones. One month postoperatory, the patient was asymptomatic, without additional visual impairment. The hormonal assays indicated persistent high FSH serum level, normal thyroid hormones, testosterone, LH and PRL levels, with low cortisol serum level. The therapy was supplemented with Bromocriptine (10 mg daily) and with undecanoate testosterone (80 mg daily p.o.). Three months postoperatory, the FSH serum level was persistently high and PRL level was under the normal values. LH and thyroid hormones serum levels were normal and testosterone level was over the normal values.
The persistent postoperatory high FSH serum levels are suggestive for an FSH secreting pituitary adenoma. The treatment management has to be reevaluated depending on the clinical and paraclinical evolution.

P133

Cross-sex hormonal therapy in transsexuals: a survey in Munich

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Introduction
Transsexualism associates biological normality with the conviction of belonging to the opposite sex and the gender reassignment request. The therapy includes psychological, hormonal and surgical treatment. Only limited clinical studies addressing cross-sex hormonal therapy and side effects exist.
Methods
Here, we report the results of a drafted questionnaire evaluating etiological aspects and treatment outcome. The questionnaire was distributed to transsexual patients participating in the German transsexual meeting in July 2007 in Munich. Thirty-eight data sets were analysed. All patients received hormonal therapy; 70% of the male-to-female transsexuals (MFT) and 55.5% of the female-to-male transsexuals (FMT) had undergone surgery at study time point.
Results
Out of 38, 20 were MFT (average age 43.3 ± 11.5 years; average age of diagnosis 39.4 ± 11.7 years) and 18 FMT (average age 36.2 ± 8.4 years; average age of diagnosis 31.6 ± 8.4 years). All patients followed differing treatment regimes: 55% of the MFT received transdermal estrogenes, 65% oral estrogenes, 30% oral progesterone and 2% intramuscular progesterone. In FMT, 23.5% received transdermal testosterone and 94.1% intramuscular testosterone. 95% of all MFT and 100% of FMT patients reported to be satisfied with the HT. However, 25% of MFT reported symptoms such as general weakness and tiredness while 44.4% of FMT reported side effects such as acne, sudoration and depressive mood. MFT as well as FMT (55 and 61.1%, respectively) reported a significant weight increase following hormonal therapy (mean increase 6.9 ± 3.6 kg).
Conclusion
Although patients report overall satisfaction with the hormonal treatment, the preliminary data points to a high prevalence of side effects. Further analysis in a larger sample of patients will be performed to elucidate if certain subgroups are more affected than others to optimize future cross-sexual therapy concepts.

P134

Optimization of chronic disease patient management: a pilot project on Sandostatin® LAR® treatment via homecare service

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In chronic diseases patient (pat) often need continuous med treatment. In acromegaly and neuroendocrine tumors (GEP-NET), long term monthly injections of somatostatin analogues as Sandostatin® LAR® (SA®) are the treatment of choice. Problems regarding pat management and drug application might arise in not-specialized centers such as general practicioners (GP). To optimize pat' management and compliance a homecare project* was piloted with specially trained nurses who applied SA® at pat' home. Pat' informed consent and doctors' (doc) written delegation were prerequisites. Prior consulting between nurses, doc and pat took place, and GPs were informed. 3 centers included 41 pat (15 acromegaly, 25 GEP-NET, 1 thyroid-ca) from 02-10/2007.153 applications were conducted (Ø 3.7, doses: 15×30 mg, 21×20, 4×10). Fifty percent of pat received SA® for the first time. The project was evaluated by an independent survey interviewing 16 pat, 9 doc and 10 nurses. All pat were very satisfied. For 69% the well organised service was the most important benefit, for 44% the time saving. Eighty percent of the doc were very satisfied as the program showed benefits regarding pat' satisfaction, time savings and low drop out rates (7/41 pat). Reasons for pat not participating were good contact, long experience and proximity to GP/clinic. Nurses were characterized as professional, friendly and well-organized. Nurses mentioned no or little problems with project start or med applications. Cooperation with doc was good. Training was found to require improvements especially regarding possible side effects and disease background. All participants recommended continuation of the homecare service. Homecare service is an innovative concept optimizing med care in chronic disease. Benefits are time saving for doc and pat, simplification of processes, professional injection of med resulting in higher pat' satisfaction and compliance.

P135

Acute neurological onset in primary hyperaldosteronism

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Among hypertensive population, almost 10% have primary hyperaldosteronism. In these cases, most abnormalities are related to hypokaliemia with progressive or acute onset of the symptoms.
We present the case of 34-year-old male patient, without significant pathological history, with a sudden neurological episode, dominated by tetraplegia and mild elevated blood pressure. Extremely low values of serum potassium are found. Diabetes mellitus is also discovered. High levels of aldosterone (3 times above normal) are found. The computed tomography revealed a left adrenal tumor of 1.3 by 1 cm diameters. The neurological signs are remitted in 2 weeks after administration of spironolactone and hydro-electrolytic solutions. Surgical resection is performed and hystopathological diagnosis of Conn's syndrome is confirmed. Normal postoperative serum potassium, aldosterone and glucose are found.
Particularities of the case are: acute onset of hypokalemia with neurological complications, completely reversible with potassium supplements and excellent evolution after surgery, no other medication being necessary. Even if some typical features of Conn's syndrome are present, our case is a male, and the disorder is seen more frequently in women.

P136

Tumors of the clivus: various entities requiring different therapies

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Introduction
Besides an incidental finding on MRI, headache, cranial nerve palsy, or pituitary insufficiency may lead to the diagnosis of a clivus tumor. Hormone secreting pituitary adenomas mimicking a clivus tumor can be easily identified by hormone testing, however, have to be considered prior to surgical procedures.
Methods
Within the last 3 years, 9 patients were transferred to us with the diagnosis of a clivus tumor for surgical therapy.
Results
Clinically, headaches led to initial MR imaging in 7 cases, 2 patients presented with VI nerve palsy. Two patients were diagnosed with prolactinomas prior to surgery, one patient had a mucocele of the sphenoid sinus with erosion of the clivus, another patient was diagnosed with a chondrosarcoma. In one patient a fibrous dysplasia of the clivus was found, 4 patients suffered from clivus chordomas. The prolactinoma patients were managed with dopamine agonists, all other patients were operated on via the transsphenoidal route, leading to complete resection of the mucocele and partial removal of the other entities. In cases of chondrosarcoma and chordoma, a proton beam radiotherapy was recommened.
Conclusion
In every patient, a careful exclusion of a prolactinoma is mandatory prior to a surgical intervention. The authors also recommend a preoperative CT study in osteolytic skull base tumors to exclude a (although rare) fibrous dysplasia. Depending on histology, a proton beam radiotherapy has to be considered in cases of chordomas, chondromas, or chondrosarcomas.

P137

A novel compound heterozygous mutation of the aromatase gene in adult man: new insight into the role of estrogen on gonadal development

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We present a novel heterozygous compound inactivating mutation of the CYP19A1 (P450arom) gene in a 26-year-old 46-XY Caucasian Italian male leading to aromatase deficiency. The patient phenotype resembled those observed in the other estrogen-deficient patients: tall stature with continuing linear growth, bilateral genu valgum, unfused epiphyses and delayed bone age, osteopenia. A dysmetabolic syndrome characterized by overweight (BMI 29), hyperinsulinaemia, low serum HDL and increased LDL was present. Virilization, penis and testis (15 and 20 ml for right and left testes respectively) size were normal. Right cryptorchidism was surgically corrected when he was 3-year-old. Sperm count was normal with reduced viability. Gonadal axis presented normal serum LH and testosterone, increased serum FSH and undetectable estradiol. DNA analysis: all coding exons with their flanking intron sequences of the CYP19A1 gene, along with untranslated exon I.4 and its 5 flanking region, were amplified by PCR and sequenced on a ABI Prism 3100 Genetic Analyzer. DNA sequencing of the CYP19A1 gene revealed a pattern of compound heterozygosity due to a 23 bp deletion in exon IV and a point mutation in the first nucleotide of intron IX, respectively. The heterozygous deletion would be expected to cause a frameshift with a premature stop codon at nucleotide 361 in exon IV; instead the single base mutation in the first nucleotide of intron IX would lead to an aberrant splicing of the mRNA. This new case of aromatase deficiency confirms the well-known effect of congenital estrogen deprivation on skeletal maturation and bone mineral density. Furthermore the concomitant presence of cryptorchidism described in other two men with aromatase deficiency support a possible role of estrogens in the male gonadal development.

P138

Galactocele and prolactinoma: a pathogenic association?

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Introduction
Galactocele is a rare form of cystic, benign lesion of the breast, occurring when a mammary duct becomes obstructed and over filled with milk. It usually appears in postpartum women, either lactating or not, but it is extremely rarely seen in postmenopausal women or in men.
Subject
We present the case of a 37-year-old female patient, nullipara, diagnosed with a microprolactinoma 18 months ago, and treated with bromocriptine since then (7.5 mg daily). The ultrasound of the breast showed normal aspect. The patient stopped the treatment for 2 months and resumed it when prolactin levels rose again up to 100 ng/ml. On admission in our hospital, the physical examination revealed a large, painless left breast, with no localized masses at palpation. Ultrasound scan indicated a large cyst (>10 cm), with drop shadows inside. The mammography showed a large, homogenous cyst. Aspiration of the cyst revealed a milky fluid, rich in lipid droplets, and with no bacterial growth, confirming the diagnosis of galactocele. After the aspiration, the echographic image disappeared.
Particularities of the case
1. The rarity of a galactocele in women not connected with pregnancy or lactation. 2. The possibility of hyperprolactinemia (even mild) due to the prolactinoma to induce the growth of the cyst. 3. The difficulty of etiological diagnosis based only on mammography and ultrasound.

P139

Biochemical markers of bone in coeliac disease

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Introduction
Prevalence of celiac disease (gluten enteropathy) in general population is around 1:100–300. Half of these patients are women. Most common age for diagnose is third year or third to fourth decade of life, due to mild clinical signs. In the

moment of diagnosis, 30% of patients already have lowered bone mineral density (BMD). Pathophysiologic mechanism for lowering BMD is secondary, regulative, intestinal hyperparathyroidism.
Aim
To trace changes in biochemical markers of bone, before and during the therapy, in two female patients of various age with celiac disease.
Materials and methods
Patient A: 21-year-old in generative period; patient B: 53-year-old, two years in menopause. After diagnosing celiac disease, patients were on 'gluten free' diet. Biochemical markers of bone metabolism, osteocalcin and croslaps were elevated in both patients, together with high values of parathormone and low levels of calcium in sera. This was a classic representation of secondary, regulative, intestinal hyperparathyroidism. Along with proper diet, patients were given supplementation of alphacalcidiol and calcium.
Results
During the therapy, there was significant decrease in values of crosslaps, osteocalcin and parathormone, with normalization of calcium levels in both patients. After 5 to 6 months, levels of osteocalcin in patient A and B was decreased 35.4 and 90%, respectively. Decrease of crosslaps was 41.2 and 83.5%; decrease of parathormone was 30.8 and 77.6% for patients A and B, respectively. After a year patient was diagnosed an osteoporosis and antiresorptive therapy with alendronat was induced.
Discussion and conclusion
During celiac disease there is significant increase in bone metabolism. Although there is reversibility in bone metabolism disturbances during therapy of gluten enteropathy (celiac disease), therapeutic approach differ depending on different risk factors non related to celiac disease.

P140

Prospective study of latent pernicious anemia markers in patients with type 1 diabetes mellitus

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Introduction
Patients with type 1 diabetes mellitus (DM1) present a high prevalence of autoimmune associated diseases. Recently, we described a prevalence of latent pernicious anemia (LPA) of 12.4% in DM1.
Objectives
A 5-year follow-up study of a cohort of DM1 patients in order to evaluate the evolution of biochemical markers of LPA, defined as a serum pepsinogen I (PI) concentration below normal limits.
Material and methods
One hundred and eighty six DM1 patients (93 men, aged 30±9.4 years). In 2001 and 2006, in all of them we measured the serum concentration of PI and gastrin, HbA1c, cobalamin and parietal cell antibodies (PCA).
Results
Twenty-three of 186 DM1 patients presented LPA at baseline in 2001. In 2006, 17 of these patients confirmed with low serum PI and in five the levels normalized. One patient was lost at follow-up. All those patients whose PI levels normalized in 2006 had normal gastrin concentrations and negative PCA in 2001, while 13/18 (72%) of patients with low serum PI in 2001 and 2006 had positive PCA at baseline. In 2006, PI was low in 6 new patients who presented normal PI in 2001. From these, two patients presented positive PCA and other two high gastrin concentrations. The cobalamin concentration in 2001 was similar in patients with or without LPA, but in 2006 it was significantly lower in the group with LPA diagnosed in 2001 ($P=0.007$).
Conclusions
Most DM1 patients with low serum PI concentrations present positive PCA, while in those in which PI normalize during the follow-up the PCA are negative. The prevalence of LPA in DM1 patients increases with the follow-up. The detection of low PI concentrations allows the identification and treatment of patients with low cobalamin concentrations before they develop clinical anemia and neurological complications.

P141

Monogenic form of polycystic ovary syndrome due to mutation in lamin A/C gene: case report of two sisters

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We report of two sisters with hyperandrogenism, menstrual abnormalities, and severe insulin resistance. The elder sister was seen after puberty at age 21 and she was referred for the evaluation of hirsutism and polymenorrhoea; the younger was seen earlier in life, at age 15, for the evaluation of secondary amenorrhoea. In both of them, we performed the diagnosis of polycystic ovary syndrome (PCOS) in accordance to the Rotterdam criteria. They also presented a lipodystrophic phenotype, characterized by loss of fat from the extremities, trunk and gluteal regions and excess fat deposition in face, neck, axillae and back. The two sisters started an oral treatment with metformin (1700 mg/day), that was continued uninterruptedly for 2 years. Because of the lack of improvement in clinical and metabolic pattern, pioglitazone (30 mg/day) was added to metformin, with progressive amelioration of hyperandrogenism, insulin resistance and hyperinsulinemia. Menses also improved, with restoration of a eumenorrhoeic pattern, but weight and waist circumference remained unchanged. The characteristic phenotype and the marked insulin resistance induced us to screen them for candidate genes involved in insulin signalling pathway. We detected a heterozygous missense mutation in codon 482 (R482Q) of the lamin A/C gene consistent with the diagnosis of Dunnigan-type familial partial lipodystrophy. Notwithstanding the autosomal dominant inheritance of the disease, LMNA genotyping showed that none of the alive members of the family was carrier of the R482Q mutation (father and grandmother were not screened because deceased). At the end of the analysis, we reformulated the diagnosis into PCOS secondary to Dunnigan-type familial partial lipodystrophy. This form widens the knowledge of the monogenic forms of PCOS, gives new insights into the relationships between insulin resistance and PCOS, with practical consequences on therapeutic choices.

P142

Coexistence of primary hyperparathyroidism and hyperthyroidism due to Graves' disease: case report

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The simultaneous occurrence of primary hyperparathyroidism and hyperthyroidism due to Graves' disease in the same patient is quite rare. Primary hyperparathyroidism accounts for hypercalcemia in only 1% of the thyrotoxic patients. The occurrence of hypercalcemia in thyrotoxic patients may represent a challenging diagnostic approach.
In this report, we describe the first case diagnosed in the Clinic of Endocrinology Timisoara with hyperthyroidism due to Graves' disease and hypercalcemia due to PTH-secreting adenoma.
A 55-year-old woman was referred to our clinic for investigations. She had signs and symptoms of thyrotoxicosis and Graves' ophtalmopathy. In routine laboratory investigations, hyperthyroidism and mild hypercalcemia were detected. Thyroid ultrasonography revealed a small goiter with hypoechoic parenchyma. Under thyrostatic therapy the patient became euthyroid. Further, laboratory determinations revealed maintaining of hypercalcemia and decrease of phosphoremia. Parathyroid hormone determination indicated high value. In ultrasonography, a small hypoechoic nodule was revealed in the lower posterior part of the right thyroid lobe, corresponding to right lower parathyroid gland. No complications of hyperparathyroidism were detected. The patient was operated, subtotal thyroidectomy and right inferior parathyroid adenoma removal being performed. Postoperatively, the total calcium and phosphorus returned to normal values and the clinical condition of this patient was very good.
Conclusion
The persistence of hypercalcemia in patients with Graves' disease after achieving euthyroid status raises the suspicion of primary hyperparathyroidism. The high values of PTH confirm the coexistence of these two endocrine disorders, having probably no causal relationship. Thus, early diagnosis and surgical therapy of hyperparathyroidism, before occurrence of complications, confer a good evolution and prognosis for these cases.

P143

Partial pituitary failure presenting as adrenocortical failure in pregnancy

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A 39-year-old lady presented with chest infection and was found to be hyponatraemic (114 mmol/l) during her third pregnancy. Short synacthen test (SST) revealed inappropriately low ACTH (39 ng/l) with adrenocortical failure (cortical 163, 355, 503 at 0, 30 and 60 min respectively). She was started on replacement hydrocortisone. Adrenal autoantibodies were negative. Other tests showed IGF-1 14.8 (13–50 ηmol/l), growth hormone <0.1 (<10 mU/l), prolactin 677 (<700 mU/l), oestradiol 106, LH 3.7, FSH 6.7. Thyroid function test TSH 2.61 (0.35–5.5 mU/l), Free T3 3.0 (3.0–6.5 ρmol/l), Free T4 9.7 (10.0–23.0 ρmol/l) twelve weeks postpartum. CT of adrenals was normal. MRI pituitary showed normal pituitary gland. She managed to breastfeed postpartum. The first pregnancy (emergency caesarean section, 39 weeks) was complicated by pre-eclampsia, pneumonia and vaginal bleeding requiring dilatation and curettage. In her second pregnancy, she had antenatal and postpartum bleeding but breastfed. A postpartum SST showed partial recovery of the adrenocortical function 313, 525, 612 nmol/l at 0, 30, 60 min respectively. ACTH was 20 ηg/l. Insulin tolerance test revealed partial pituitary failure with maximum cortisol of 449 ηmol/l and maximum growth hormone of 9.1 mU/l at 90 min. TRH stimulation test showed normal TSH response. The gonadotrophin stimulation test was not done as she had regular periods. She is well at present, without hormone replacement and under regular follow-up. She has been advised to take hydrocortisone at times of stress. This case illustrates the dilemmas in interpreting results, especially during pregnancy. While the initial SST indicated adrenocortical failure, the aetiology could not be determined due to pregnancy. Subsequent investigations suggest partial pituitary failure that became apparent at times of stress. Aetiology of pituitary failure remains elusive and is thought to be due to either lymphocytic hypophysitis or Sheehan's syndrome.

P144

The safety and efficacy of injectable testosterone undecanoate during routine clinical management of hypogonadism: an analysis from a long-term 'real-life' study

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Objective
This open-label, single-arm study assessed the long-term safety and efficacy of injectable testosterone undecanoate (TU; Nebido) in men with hypogonadism.
Methods
Men aged 18–75 years with hypogonadism (baseline serum total testosterone <10 nmol/l) were recruited. The study was performed under conditions designed to resemble routine clinical management: patients with underlying conditions such as diabetes mellitus and hypertension were eligible for inclusion, and variable injection intervals were permitted. The study protocol was approved by the appropriate independent ethics committees. Patients received intramuscular injections of 1000 mg TU at treatment visits separated by intervals of 6–10 weeks (after the first injection) or 10–14 weeks (after all subsequent injections). Here, we report the findings of an analysis performed at the end of the interval following the tenth injection (1.8–2.6 years after patient entry into the study). The primary endpoint of the study was the serum level of prostate-specific antigen (PSA; assessed at baseline and at alternate treatment visits). Secondary variables included trough total testosterone levels (assessed at baseline and all treatment visits).
Results
A total of 77 patients (mean (standard deviation) age, 47.8 (11.6) years) were included in this analysis. Mean PSA levels remained close to baseline throughout (baseline: 0.81 (0.78) ng/ml; before ninth injection: 1.14 (1.1) ng/ml). Mean haematocrit remained with the normal range, and treatment was generally well tolerated. Trough total testosterone levels were restored to the physiological range after the second injection and throughout the remainder of the study (baseline: 5.8 (3.0) nmol/l; before tenth injection: 13.6 (3.7) nmol/l).
Conclusions
This 'real-life' study – performed under routine clinical conditions – confirmed the safety of long-term intramuscular TU treatment in men with hypogonadism. The efficacy of treatment in restoring and maintaining physiological levels of serum testosterone was also demonstrated.

P145

Hypercalcemic renal failure in splenic sarcoidosis

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A 46-year-woman was admitted with decreased appetite, weight loss, nausea, constipation, poor concentration, cough and shortness of breath on exertion. She had long standing history of low backache and had been taking paracetamol and codeine in combination, amitriptyline, tramadol, stemetil, diclofenac, and calcichew D3 one tablet/day On examination, blood pressure was elevated, erythematous nodules seen over right shin. Initial investigations showed normocytic anemia, elevated urea, creatinine (283 umol/l) and calcium. Paratharmone was still detectable even after stopping calcichew D3 and urea, creatinine(190 umol/l) and calcium continued to be elevated even after rehydration and intravenous bisphosphonate. Imaging studies showed normal chest X ray and ultrasound of thyroid and CT abdomen and chest revealed borderline spleenomegaly with multiple coalescent nodular lesions and patchy lower lobe fibrosis in lungs. Bone marrow study showed normal cellularity. Diagnostic splenectomy was done, histopathology of which confirmed sacroidosis. Renal function improved, (creatinine 145 umol/l) and calcium returned to normal in the next 3 days.
Granuloma is the site of calcitriol and sometimes paratharmone related protein production resulting in hypercalcemia. Steroids inhibit the calcitriol production in granulomas. Hypercalcemia *per se* with its consequences on kidneys or direct involvement of interstitium by granuloma result in renal failure.
In our patient, splenectomy resulted in removal of bulk granulomas and thus calcitriol and possibly paratharmone related protein. This helped in return of normal renal function and calcium.

P146

Testosterone treatment improves muscle strength, lean mass and quality of life in prefrail and frail elderly men: results from a randomised double-blind placebo-controlled study

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Introduction
Testosterone (T) improves muscle strength in hypogondal patients. It is unclear if testosterone has similar effects in frail elderly men with low T. We conducted a randomised double-blind placebo-controlled parallel group study to determine the effects of T on muscle mass and strength, physical function and quality of life in frail elderly men from the general population.
Methods
Two hundred and sixty-two prefrail and frail elderly men (criteria of Fried *et al.* 2001), mean age (range) 74 (65–89) years received testosterone (25–75 mg/d) or placebo gel for 6 months. Outcome measures included muscle strength {(primary end points – isometric peak torque, knee extension (EIMPT) and flexion (FIMPT)}, physical function tests, lean mass (DXA) and quality of life {aging males' symptom (AMS) scale}. Ethical approval was obtained from the Central Manchester research ethics committee.
Results
T at baseline was 10.9 ± 3.1 and 11 ± 3.2 mean (S.D.) nmol/l in active and placebo groups. T increased to 22.9 ± 10 nmol/l in the active with no change in placebo group (11.3 ± 5.2 nmol/l). EIMPT improved by 6% ($P=0.042$) in active and 3% ($P=0.17$) in placebo group. Men who reached target testosterone levels during treatment achieved higher EIMPT (10% increment) versus those that did not (2%). Physical function tests improved but did not reach statistical significance. Somatic subscale domain of AMS improved; adjusted difference (95%CI) for active vs. placebo group was -1.2 (-2.4 to -0.04). Lean mass increased (1.07 kg, $P<0.0001$) in the active versus placebo group.
Conclusions
Treatment with transdermal testosterone for 6 months, leads to improvement in lean mass, muscle strength and physical symptom – related quality of life in prefrail and frail elderly men.

P147

Severe nonalcoholic fatty liver disease in a young woman with polycystic ovary syndrome and insulin resistance: case report

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Women with polycystic ovary syndrome (PCOS) and insulin resistance have an increased risk of developing many of the consequences of the metabolic syndrome. Obesity, but in particular metabolic syndrome seems to be the main cause of nonalcoholic fatty liver disease (NAFLD) so, it is not surprising that NAFLD is very common in patients with PCOS. We present the case of a 23-year-old woman diagnosed with PCOS and insulin resistance in our Endocrinology Clinic (irregular menses, hyperandrogenemia, polycystic ovary morphology and increased ovarian volume on pelvic ultrasound, high levels of fasting insulin and after glucose administration). The clinical approach revealed a body mass index (BMI) of 34 kg/m^2 with waist circumference of 108 cm, no evidence of hirsutism and a blood pressure of 180/120 mmHg. The lab tests showed elevated serum aminotrasferase levels (ALT = 203 U/l, AST = 145 U/l), normal alkaline phosphatase and a high level of fibrinogen (525 mg/dl). She had no risk factors for viral hepatitis and denied having any history of alcohol use. Further work-up of the abnormal liver enzymes revealed negative serologic studies for hepatitis B and C, negative test results for antimicrosomal and antinuclear antibodies, and a normal plasma ferritin level. The abdominal ultrasound showed hepatic steatosis. We excluded other possible causes for secondary hypertension. We suggest that evaluation for liver disease should be considered at a much earlier age in women with PCOS and components of the metabolic syndrome.

P148

A case of hungry bone syndrome during therapy with methimazole for hyperthyroidism

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We describe a case of severe hypocalcemia after methimazole treatment for Graves disease, which closely resembles classic hungry bone syndrome.
A 41-year-old woman with a history of Graves disease presented to our Internal Medicine ward with tachyarrythmia. Laboratory data showed a severe hyperthyroidism, an ecocardiography demonstrated a dilated cardiomyopathy. The patient was given beta-blockers, low molecular weight heparin, and methimazole (30 mg/day). One month after the start of treatment she was readmitted to hospital with dyspnea, tachyarrhythmia, and hypotension. Few hours after admission the patient experienced a tetanic crisis. Total serum calcium was 1.83 mmol/l (normal, 2.10–2.55 mmol/l), and ionized calcium was 0.84 mmol/l (normal, 1.19–1.29 mmol/l). The patient had low thyroid hormone levels. Methimazole was stopped and levothyroxine was started at a low dose. In addition, the patient was given IV calcium gluconate (720 mg elemental calcium per day), magnesium sulphate (2 g per day), and potassium chloride (30 mEq per day), following a complete resolution of tetanic symptoms and normalization of serum and ionized calcium.
It is assumed that hypocalcemia was a complication of medical therapy for thyrotoxicosis. Thus far, only a few cases of hypocalcemia after medical treatment of hyperthyroidism have been reported. To the best of our knowledge, this patient is second case of hungry bone syndrome following drug treatment of thyrotoxicosis in the literature. We suggest following serum calcium levels for the first few weeks of methimazole therapy in hyperthyroid patients.

P149

Giant myelolipoma in a patient affected by 17-α-hydroxylase deficiency and β-thalassemic trait

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Myelolipomas are rare benign tumours resulting from myeloid and adipose mature cells proliferation, both elements have a clonal origin from a common precursor. Myelolipomas predominantly involve the adrenal gland but may develop in extra-adrenal sites. They are hormonally inactive but may coexist with active diseases such as adrenogenital syndrome. They are often asymptomatic but rarely they cause symptoms due to their size or spontaneous retroperitoneal haemorrhage. The myelolipomas, as a response to chronic hypoxia, are frequent in more severe forms of thalassemias but not in traits. 17-α-hydroxilase deficiency is a rare cause of congenital adrenal hyperplasia due to defect in cytocrome P450C17 with elevated levels of ACTH leading to hypertension, hypokaliemia, and sexual development abnormalities. We report a case of a 48-year-old patient with primary amenorrhea who was diagnosed with a 17-α-hydroxylase deficiency. Since diagnosis, desametasone, hydrocortisone, antihypertensive and EEPP therapy was prescribed but the patient assumed it discontinuously. Mycrocytic hypochromic anemia was present due to a β-thalassemic trait. The erythropoietin response to anemia was near to the lowest limit, although chronic renal failure was present. An abdomen MRI documented two bilateral voluminous masses at the adrenal site. Left adrenalectomy was performed. The histological examination of the mass recognized a myelolipoma. This is the only case in literature that describe the concomitant presence of myelolipomas and β-thalassemic trait in a patient with 17-α-hydroxylase deficiency. Some hypothesis on the pathogenesis of adrenal myelolipoma can be made. Perhaps, the patient had periodical high ACTH levels due to the low adherence to suppressive therapy and the chronic adrenal gland stimulation resulted in myeloid metaplasia of adrenocortical cells. It is well known that ACTH has stimulatory effect on erythropoietin production. These two actions can be responsible of the myelolipoma growth in a patient with β-thalassemia trait.In this case the chronic stimulating ACTH effect strengthened that of a mild anemia.

P150

Subclinical Cushing's syndrome (CS): role of ^{131}I-iodomethylnorcholesterol scintigraphy in predicting the evolution of the disease

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Subclinical CS, mild hypercortisolism without overt clinical manifestations, is the most frequent (5–8%) hormonal abnormality detected in patients with secreting adrenal incidentalomas.
Unclear clinical features and mild hypercortisolism make the diagnosis problematic, although laboratory criteria have recently been reviewed. In these cases, scintiscan is a central tool to define the adrenal functional activity.
A 60-year-old man was referred to us because of hypercolesterolemia and hypertension treated by calcium-antagonists and alpha-lytics.
CT performed because of suspected kidney stones showed a 4 cm right adrenal mass with radiological features indicative for adenoma.
The adrenal 'incidentaloma' was evaluated for possible hypersecretion: screening tests for primary hyperaldosteronism and pheochromocytoma were negative.
Circadian rhythm in serum cortisol concentration and urinary free cortisol (UFC) were normal. Plasma ACTH concentration was low (7 pg/ml). Overnight dexamethasone 1 mg suppression test (DST) was uncertain: serum cortisol at 8 AM was 21 ng/ml.
^{131}I-iodomethylnorcholesterol scintigraphy showed unilateral uptake on the side of the adrenal mass (concordant uptake).
Because of the borderline biochemical findings and subclinical presentation, careful observation associated with treatment of metabolic syndrome was preferred. A new evaluation was planned after 6 months. At that time a marked increase of urinary free cortisol, elevated midnight cortisol and nonsuppressible cortisol (60 ng/ml) after 1 mg DST were observed. ACTH was less than 5 pg/ml. Abdominal CT was unchanged. Furthermore, in spite of medical therapy, a worsening in metabolic and blood pressure control was detected. Therefore the patient underwent surgery. Postsurgical hypoadrenalism is currently treated by HC.
This case confirms the usefulness of radiocholesterol scintigraphy in detecting adrenal hyperfunction in patients with subclinical CS. An incidental mass showing unilateral concordant radionuclide uptake in spite of the absence of clear clinical and laboratory findings is predictive of a possible evolution in overt CS and makes necessary a close follow up.

P151

Two unusual cases with pancytopenia associated with Sheehan's syndrome

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Introduction
Sheehan's sydrome was known varying degrees of hypopituitarism due to postpartum ischemic necrosis by different etiologies. We report two case who are presented with pancytopenia which is unusual and rarely observed an important clinical finding associated with Sheehan's syndrome.
Case 1
A 57-year-old woman has complaints of malaise, fatigue, and dyspnea without lymphadenopatia and hepatosplenomagaly. She had excessive bleeding after last delivery at age 36 with subsequently lactation failure and amenorrhea. Endocrinologic evaluation revealed hypopituitarism, and her complete blood count showed anemia, leukopenia and thrombocytopenia due to decreased hematopoiesis with hypocellularity in bone marrow. Sheehan's sydrome is treated with hormone replacement therapy included prednisolone and L-thyroxine. After three monts of replacement, hematologic findings is fully recovered.
Case 2
Seventy-two-year-old woman is presented with hypoglycemia and pancytopenia, and her physical examination and history of a previous massive postpartum hemorrhage suggested Sheehan's syndrome. Hormone profiles revealed hypopituitarism, magnetic resonance imaging of pituitary gland showed empty sella. After a week of prednisolone and L-thyroxine replacement therapy, hematologic findings recovered completely.
Conclusion
Pancytopenia is rare complication in association with Sheehan's sydrome, and devoleped due to loss effect of pituitary hormones on metabolic reactions to hematopoiesis related to hypopituitarism. It was recovered with replacement therapy within a week to 3 months. The diagnosis of Sheehan's syndrome can be delayed due to slow progression. Obstetric history, menstruation and lactation status must be questinoted and included careful management to avoid postpartum complications.

P152

Pituitary carcinoma presenting as Cushing's disease

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At our department of Endocrinology a 44-year-old patient with typical clinical signs of Cushing's disease presented. Diagnostic procedure showed an ACTH-producing adenoma of the pituitary gland, so the patient underwent transsphenoidal surgery. Afterwards, hypercortisolism persisted and was treated with Ketoconazole. After a short period of time a pituitary hemorrhage occurred and resulted not only in a complete remission of Cushing's symptoms, but also in hypopituitarism. One year later, the symptoms reoccured and MRI showed a regrowth of the pituitary adenoma. Surgery was repeated and this time histology showed a pituitary carcinoma. In the following years, therapy strategies included radiatio, adrenectomy and chemotherapy but these could not prevent the development of liver metastases and tumour progress, so the patient died 6 years after the onset of the disease.

P153

Hypoglycaemia following gastric banding

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Introduction
Recently, noninsulinoma pancreatogenous hypoglycaemia syndrome (NIPHS) has been described following Roux-en-Y Gastric Bypass Surgery in morbidly obese patients. It has been proposed that hypoglycaemia might be a consequence of a failure to adaptively decrease insulin secretion after surgery. The authors present a case of a morbidly obese patient with severe hypoglycaemias beginning 3 months after gastric banding surgery.
Case report
A 60-year-old patient, with a long evolution history of morbid obesity (BMI 52.2 kg/m^2) and HBP since the age of 28, was submitted to gastric banding in December/2006. He lost about 30 kg in the 6 months following. Three months after surgery, clinical and laboratory hypoglycaemias were detected, sometimes with sudden loss of consciousness. The fasting test was performed and blood test results revealed: glucose 34 mg/dl; insulin 19.8 uUI/ml; Cpeptide 1.53 ng/ml. An abdominal helicoidally CT scan showed a 1.9 cm tumour in the head of the pancreas and a 1.1 cm tumour in the neck of the pancreas. Selective intraarterial injection of calcium gluconate was performed; there was a significant increase in serum insulin concentration after injection in gastroduodenal and splenic arteries, but not after injection in mesenteric or hepatic arteries. After medical therapy with oral diazoxide, he was submitted to proximal pancreatectomy on 07/07/24. The postoperative course was complicated by pancreatic fistula, retroperitoneal abscess and transient hyperglycemias. Histopathology revealed an insulinoma with well-defined contours. Three months after surgery the patient is asymptomatic and euglycemic.
Conclusion
Hypoglycaemic state in morbidly obese patients following bariatric surgery may occur as a result of endogenous hyperinsulinism. In the presence of hypoglycaemia, it seems important to establish a differential diagnosis between insulinoma and NIPHS.

P154

Serious hypocalcemia as the first display of coeliac disease of the Down syndrome patient

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The coeliac disease is the autoimmunity disease of small intestine, which is the immune response result to various proteins of cereals, especially wheat gliadin. in adulthood it often manifests with various symptomatology, which includes metabolic osteopathy arising on secondary hyperparathyreosis.
There is casuistic model of 24-year-old patient with Down syndrome and documented autoimmunity thyroiditis, where the coeliac disease manifested by serious hypocalcemia with patologic fractures of both femur necks.
In our endocrinology ambulance, she was consulted because of the legs swelling with the suspicion for the insufficient thyroidal hormones substitution. She had bones pains for 1 year sharply worsening last month and finished to total immobility. During laboratory screening there was found serious hypocalcemia (Ca: 1.30 mmol/l, Ca^{2+}: 0.94 mmol/l). Following examinations confirmed serious bones impact (*T* score – 3.54, max in area of L4 – 5.155) and fractures of both femur necks.
Considering to known association between M. Down and coeliac disease there was added the antigliadin antibodies examination, antibodies against tissue transglutamilation and endomysial antibodies, which were highly positive and confirmed supposed malabsorption syndrome diagnosis.
At present day, the pacient adheres antigluten diet, serum concentrates of calcium are through permanent supplementation (Ca, vitamin D) near bottom limit of reference range and there is planned femur necks fractures solution.
There is frequent association between coeliac disease and other autoimmunity diseases particularly autoimmunity thyroiditis. The relationship between coeliac disease and autoimmunity thyroiditis was already described 30 years ago and on the base of present knowledges there is recomended according some authors to make screening for coeliac disease of the autoimmunity thyroiditis pacients and vice versa.

P155

Brown tumor of the mandible and osteitis fibrosa cystica in hyperparathyroidism: a rare disease mimicking an osseous metastasized malignancy

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Background
Osteolytic metastases of the bone occur in several malignant tumors. Beside osseous metastases, endocrine diseases like primary hyperparathyroidism can mimic osteolytic metastases.
Case report
A 53-year-old woman was sent to the hospital by an oral surgeon with a worsening of her general condition, fatigue, a painful tumor of the mandible, diffuse bone pain and the diagnosis of a suspected metastasized malignancy. Laboratory testing showed a severe hypocalcaemia and a massive elevation of parathyroid hormone levels.

A bone scintigraphy showed an enhancement in the mandible as well as several other lesions in the costae, the sacrum and the right ankle. Histological examination of a tumor biopsy indicated a giant cell tumor. CT scans of the abdomen and thorax showed calcification of the stomach and diffuse calcifications of the kidneys, but no tumor. A cervical sonography showed a nodal lesion at the lower part of the right thyreoidal lobe consistent with an enlarged polar body. In synopsis of all diagnostic findings, the diagnosis of a primary hyperparathyroidism with so called 'brown tumor' of the mandible and osteitis fibrosa cystica was made. The parathyroid gland was surgically resected and histological examination showed an adenoma of the parathyroid gland. Three months after surgery, a complete remission of the hyperparathyroidism as well as a regression of the tumor of the mandible and the skeletal lesions were documented.
Discussion
It has been demonstrated here that multiple osteolytic lesions and hypocalcaemia do occur not only in metastatic malignant tumors but are also found in endocrine diseases like primary hyperparathyroidism. Therefore, a careful diagnostic workup is essential in ensuring an optimal treatment. As in the present case, diagnosis is sometimes complicated due to the clinical appearance and histological differentiation between a giant cell tumor and the so called 'brown tumor' associated with primary hyperparathyroidism.

P156

Consequences of childhood onset growth hormone deficiency (COGHD) in cases of familial hypopituitarism

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COGHD may be of genetic origin associated with deficiency of other pituitary hormones. Non treatment with GH in these cases can cause metabolic and other disturbances. The aim of this research was examining consequences of non treated COGDH in three sisters with familial hypopituitarism. Three sisters 60-, 59- and 54-year-old with familial hypopituitarism (GH,TSH, gonadotropin and prolactin deficiency) which was diagnosed 46 years before were examined. Substitution therapy performed only with thyroid hormones and gonadal steroids. We examined anterior pituitary hormones, body mass index – BMI, body composition by BIA, lipids, OGTT with glycemia, insulin and C-peptide, bone metabolism and densitometry by DXA. Levels of pituitary hormones confirmed above mentioned deficiency and good substitution of thyroid deficiency. Therapy with gondal steroids was interrupted some years ago. Low level of IGF-I (<0.25 ng/ml) in all of them confirmed GHD. BMI and body composition were normal in all. High level of total cholesterol, LDL, triglycerides and low level of HDL were present in all of them. OGTT excluded disturbances of carbohydrate metabolism. Bone markers showed accelerated bone metabolism only in the oldest sister (osteocalcin 51.5 ng/ml, β-crosslaps 932 pg/ml). Two sisters had osteoporosis (T score: -2.76 in the oldest and the second oldest -4.2), and the youngest one had osteopenia with T score -2.4.
Conclusion
Non treated COGDH in familial hypopituitarism can cause disturbances in lipid and lipoprotein metabolism, bone mineral density and bone metabolism without disturbances of carbohydrate metabolism, obesity and pathological body composition.

P157

Giant prolactinoma: what is the best therapy?

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Giant prolactinomas are uncommon, with some individual case reports described in the literature, but with few series documenting treatment outcomes. They represent a therapeutic challenge, since restoring normoprolactinemia, eugonadism and reducing tumour size may not be realistic goals. Specific complications may also arise during treatment that change the initial management plans. The authors describe a case of a 28-year-old male with visual impairment and behavioural changes. The diagnosis of a macroprolactinoma was made, characterized by a lesion with 76 mm of larger diameter, supraselar extension, large anterior-superior cystic component and posterior-inferior solid component in relation with vascular and neural structures of the skull base. The endocrine study revealed prolactin levels of 158 700 uUi/ml (58–254), without associated hypopituitarism. A craniotomy was performed, but relapse of the cystic component in the first year of follow-up led to reintervention. Histologically the tumour was densely granulated, with strong diffuse immunostaining for prolactin, Ki-67 labelling index of 7% and p53 immunoreactivity of 42%. Bromocriptine was prescribed in a maximum daily dose of 45 mg, and a 92% reduction in prolactin levels was attained. Despite reduction in the cystic component, there were no significant changes in the solid component after treatment. Particular therapeutic questions arise. First, restoring eugonadism and normoprolactinemia is not feasible and the involvement of critical neural structures represents the major concern, but surgical approach of the solid component is difficult. High doses of bromocriptine or cabergoline may be associated with long term side effects. There is still the possibility of radiotherapy, with its known consequences. Second, this clinical course and the high Ki-67 labelling index associated with p53 staining alert to the possibility of a tumour with aggressive behaviour and malignant potential.

P158

Does cyproterone acetate promote multiple meningiomas?

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Multiple meningiomas are rare benign tumors (1.5% of all meningiomas). They are either sporadic or associated with neurofibromatosis. Their long term morbidity is high due to the frequency of surgical procedures needed to overcome the absence of efficient adjuvant therapy. We present a cohort of patients in whom we strongly suspect cyproterone acetate to be responsible for the development and progression of multiple meningiomas.
Patients and methods
We report 9 female patients (33–62 yo, mean: 46 yo) with multiple meningiomas (2 to 11) without any clinical evidence of neurofibromatosis. All patients were treated with cyproterone acetate (50 mg/day) for various indications for a time period ranging from 10 to 20 years.
Results
A rapid onset of clinical symptoms was observed in 6 patients with rapid decreased visual acuity in 5 patients, suggesting rapidly progressive meningiomas. Lesions were preferentially located on the skull base. Cyprotenone acetate was stopped at the time of diagnosis in 2 patients. Six patients were followed radiologically for a period exceeding 5 months (8 to 81) before treatment withdrawal. A significant increase in tumor size and/or the development of new lesions was observed in all cases. In six patients, the follow-up period after treatment withdrawal was more than 5 months (5 to 32 months, mean: 17 months) and no clinical nor radiological progression was observed.
Discussion
We strongly suspect cyproterone acetate as a promoting factor in the development of multiple meningiomas in concert with a particular endocrine status. We could face a particular histopathologic entity considering the preferential skull base location of lesions and the unusual absence of progression after treatment withdrawal.
Conclusion
Although undescribed in the literature, the possibility of a relationship between multiple meningiomas and cyproterone acetate, needs to be further investigated.

P159

How the workup of low renin hypertension lead to the diagnosis of SIADH

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A patient was referred to us because he had hypokalemic hypertension and no detectable blood concentrations of renin and aldosterone. In addition, this patient had an adrenal mass. Since he was hyponatremic, he was put on fludrocortisone. At presentation, we found that the patient was normonatremic and hypokalemic and had a high sodium to urinary sodium to potassium to urinary potassium (SUSPUP) ratio. But review of older laboratory results showed an inadequate high urinary concentration of sodium and a very low SUSPUP ratio. Hormonal studies revealed that the patient had undetectable low renin and aldosterone levels. However, pro-brain natriuretic peptide (proBNP) and copeptin as a measure of vasopressin were elevated to an astonishing degree. The diagnosis of a

syndrome of inadequate secretion of antidiuretic hormone (SIADH) was made. A computed tomography scan of the thorax showed a tumor in segment 7 and 8. The histology gave the diagnosis of a mall cell lung cancer and staining of the biopsy samples to copeptin were positive.
We conclude that SIADH may present as a form of low renin hypertension and that the use of modern markers such as proBNP and copeptin are useful in establishing the diagnosis of SIADH. We also conlcude that the SUSPUP ratio may help to distinguish between mineralocorticoid excess and other form of hypertension.

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Clinical efficacy of intramuscular versus oral testosterone undecanoate in adult men with hypogonadotropic hypogonadism

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Aim
To evaluate the clinical efficacy of androgen replacement therapy with oral or intramuscular (i.m.) testosterone undecanoate (T.U.) in male patients with central hypogonadism.
Patients and methods
We retrospectively evaluated 40 patients with hypogonadotropic hypogonadism: 29 with pituitary tumors or craniopharyn-giomas, 11 with non-tumoral hypogonadism (median age 47 years, range 20–62), before and after androgen replacement therapy, with the approval of the local Ethical Committee. We evaluated the sexual dysfunction (as declared by the patient), serum testosterone, haemoglobin, hematocrit, cholesterol, triglycerides (measured with commercial kits in venous blood sampled at 8.00–9.00 am).
Results
In 28 patients treated with oral T.U. (median dose 120 mg/day in 3 doses) for 6–24 months, the morning serum testosterone raised from 0.21 ± 0.4 ng/ml (median $\pm$ standard deviation) to 1.01 ± 1.37 ng/ml ($P < 0.01$), but was normal only in 2 patients (7%) (normal values 2.41-8.27 ng/ml). In 17 patients treated with 1 g i.m. T.U. at 0, 6, then 12 weeks intervals, evaluated at 1.5–10 months (median 4.5 months) from the therapy initiation, serum testosterone raised from 0.62 ± 0.77 ng/ml to 5.24 ± 4.2 ng/ml ($P < 0.01$). It was normal in 12 patients (70.5%), low in 1 (5.8%) and high in 4 patients (23.5%). The sexual dysfunction improved in 7/15 patients (46%) on oral T.U. and in 8/10 patients (80%) on i.m. T.U ($P =$ NS). Hematocrit increased to higher values in patients on oral T.U (from $37.5 \pm 3.3\%$ to 41.9 ± 4.02, $P < 0.01$) than in patients on i.m. T.U. (from $38.5 \pm 4.7\%$ to $41.1 \pm 3.9\%$, $P < 0.01$), $P < 0.01$, in none over 50%. Serum cholesterol and triglycerides did not change significantly after any of the T.U. treatments.
Conclusion
Morning serum levels of testosterone were normal and the sexual function was improved in most patients with central hypogonadism treated with i.m.T.U., but only in few patients treated with oral T.U.

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Coincidence of a diaphragma sellae meningioma and two different pituitary adenoma subtypes in a single intra- and suprasellar lesion

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Objective
Despite a wide variety of differential diagnosis, modern MRI imaging usually enables a good preoperative evaluation of the aetiology in most cases with scull base and sellar lesions. However, in some cases MRI visualization alone may also be misleading with a consecutive need to adapt intraoperative strategies.
Clinical presentation
The case of a 67-year-old male patient with the history of a bacterial meningitis, visual deterioration and the onset of diabetes mellitus is presented. MRI imaging demonstrated an intra- and suprasellar lesion of about 22 mm in diameter compressing the optic chiasm. The sella floor was enlarged. Visual acuity was 0.1 and 0.06 with concentrically narrowed visual fields. From a neuroendocrinological standpoint IGF-1 was elevated consisted with the diagnosis of a large GH-secreting macroadenoma and acromegaly.
Intervention and diagnosis
The lesion was operated by a transsphenoidal approach with typical pituitary adenoma tissue, although with a somehow inhomogeneous texture, found intraoperatively. However, the diaphragma sellae did not descent, despite the dominant suprasellar tumor parts on preoperative MRI imaging. Moreover, the diaphragma sellae was completely intact. Intraoperative high-field MRI imaging confirmed a large suprasellar tumor remnant with an already empty sellae beneath. Extending the transsphenoidal approach, a firm, partial calcified tumor originating from the diaphragma itself, was resected completely followed by an extensive reconstruction of the sellar floor.
Histopathologically the intrasellär parts of the lesion were composed of a sparsely granulated GH-secreting pituitary adenoma and a distinctly separated non-secreting adenoma with immunohistochemical expression of LH and FSH again anatomically separated by the diaphragma sellae from the suprasellar part of the lesion constituting a typical meningioma.
Conclusion
We present the rare coincidence of three immunohistochemically different and clearly separated scull base tumors with acromegaly mimicing a single intra- and suprasellar tumor on preoperative MRI imaging.

P162

Etiology of patients attending an outpatient clinic of a tertiary care centre for primary amenorrhoea: a retrospective study

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Introduction
Primary amenorrhoea is defined as the absence of spontaneous menses by 16 years of age.There have been few, if any detailed reports of primary amenorrhoea from India.
Aim
To assess the clinical profile and aetiology in patients presenting with primary amenorrhoea attending a tertiary care hospital in India.
Materials and methods
A retrospective study was undertaken of patients presenting to a tertiary care institution over five years. Data was retrieved from medical records. Chief presenting complaint/s, associated symptoms, height, dysmorphic features, mental subnormality, pubertal features were noted. Hormonal and sex steroid assay, pelvic ultrasound and karyotyping results noted. CT and MRI scans were done wherever indicated and final diagnosis noted.
Results
There were 207 patients in our study with average age of presentation being 18.3 years, ranging from 16 to 31 years. One hundred and thirty-four patients presented with primary amenorrhoea as their chief complaint (64.7%), others with failure to gain height (6.7%), lack of/delayed appearance of secondary sexual characteristics (26.7%) and obesity (1.9%). Frank mental retardation was seen in 5 and poor scholastic performance in 13. Gonadal dysgenesis was the commonest diagnosis (39%) followed by hypogonadotrophic hypogonadism (22.3%), hypothyroidism (6.2%), Mullerian agenesis (4%), genital tuberculosis (2.8%), testicular feminization syndrome (2.4%) and other miscellaneous causes.
Conclusion
As compared to previous series from western data, our data shows that the average age of presentation is considerably later, mental subnormality is less and possibly aetiology is different. Racial and environmental differences may play a role in these as well as the fact that ours is a referral unit.

P163

Clinical presentation of a patient with pseudohypoparathyroidism type 1a (Albright syndrome)

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Background
Pseudohypoparathyroidism (PHP) is a heterogeneous group of disorders characterized by hypocalcemia, hyperphosphatemia, increased serum concentration of parathyroid hormone (PTH) and insensitivity to the biological activity of PTH.
Case report
We present the case of a 38-year-old man admitted to the Neurology Clinic in November 2007 for a witnessed grand mal seizure that resolved without

intervention. Patient had a history of mental retardation, but no history of seizures in the past. On admission vital signs were stable and the physical exam showed a positive Chvostek sign, obesity and a characteristic set of skeletal abnormalities including short stature, round face, short neck and short 4th and 5th metacarpals. Relevant laboratory findings: calcium 1.56 mmol/l (*N* 2.1–2.55), phosphorus 1.51 mmol/l (*N*: 0.74–1.52), PTH: 134 pg/ml (*N*: 15–65), urinary calcium: 0.01 mmol/d (*N*: 2.5–8), urinary phosphorus: 43.1 mmol/d (*N*: 35–80). CT of the head showed calcifications of the basal ganglia and multiple calcifications of the soft tissues, electrocardiogram revealed prolonged QT interval and the ophtalmological exam showed bilateral cataract. The patient had an impaired mentation with an IQ of 55.
Discussion
Based on the examinations above, we interpreted the case as a typical presentation of pseudohypoparatyroidism type 1a (giving the striking phenotypic characteristics). This autosomal dominant genetic disease has a prevalence of 3.5 cases per 1 million people. The mainstay of treatment is vitamin D metabolites, such as calcitriol, and calcium. The goals of therapy are to maintain serum calcium levels within the reference range to avoid hypercalciuria and to suppress PTH levels to normal. Our patient also needed neurological medication for the epileptic seizures. 0.00019) than weight of examined patients ($P=0.632958$).

Comparative endocrinology

P164

c-AMP production in *in vitro* VIP- and PACAP-induced adrenal secretion of the lizard *Podarcis sicula*

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The action of the regulatory neuropeptides vasoactive intestinal polypeptide (VIP) and pituitary adenylate cyclase-activating polypeptide (PACAP) carries out through the shared receptors $VPAC_1$ and $VPAC_2$ and the exclusive PACAP receptor PAC_1, all of which are G protein-coupled receptors. Formerly, we showed that the administration of both peptides enhance catecholamine, corticosterone and aldosterone release from adrenal cell co-culture. Further, the distribution of VIP and PACAP receptors in the adrenal glands of the Italian wall lizard, *Podarcis sicula*, was previously demonstrated: $VPAC_1$ was found within steroidogenic tissue, $VPAC_2$ and PAC_1 within chromaffin tissue. In the present study we investigated the c-AMP signaling pathway involved in the VIP and PACAP-induced secretion of adrenal cell co-cultures. Using $VPAC_1$ antagonist [Ac-His_1, D-Phe_2,Lys_{15},Arg_{16}]VIP-(3-7),GH-releasing factor-(8-27)-NH_2 ($VPAC_1$-A), the PAC_1 antagonist PACAP6-38 (PAC_1-A) and $VPAC_2$ immunoneutralized cells, in *in vitro* VIP or PACAP treated adrenal cell co-cultures, we showed that the VIP- and PACAP-induced steroid and peptide hormone secretion is carried out through the activation of selective receptors which promote, in time dependent manner, the production of c-AMP: after 1 h of PACAP/$VPAC_1$-A/$VPAC_2$ immunoneutralized cells treatment, c-AMP production is 250% in relation to control. After 3 h of stimulation with VIP/PAC_1-A/$VPAC_2$ immunoneutralized cells, the c-AMP production was increased of 281% compared with control. After 24 h of treatment neither VIP nor PACAP was able to stimulate c-AMP production. Further, we showed that VIP enhances c-AMP production through the binding of the $VPAC_1$ receptor and PACAP increases c-AMP production through the interaction with the PAC_1 receptor exclusively. Thus, our investigation showed that the activation of adenylate-cyclase is required to permit the *in vitro* VIP and PACAP stimulation and PACAP is faster than VIP to induce c-AMP production in lizard adrenal cell secretion.

P165

Chemokine CXCL10 gene polymorphisms in Addison's disease

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Background and aims
CXC chemokine ligand 10 (CXCL10) also known as chemokine interferon γ inducible protein (IP-10) is a CXCR3 chemokine belongs to a group of structurally related molecules, that induce the chemotaxis of diverse leukocyte subtypes including activated T-helper 1 lymphocytes, natural killer cells and monocytes. In patients with Hashimoto's thyroiditis, type 1 diabetes mellitus and Graves' disease high levels of CXCL10 ligand in serum have been found. Therefore, we investigated the role of CXCL10 gene polymorphisms in patients with Addison's disease (AD) and in patients with Graves' disease (GD).
Materials and methods
Patients from Germany with Addison's disease ($n=174$), Graves' disease ($n=171$) and healthy controls (HC: $n=285$) were genotyped for the CXCL10_89 and for the CXCL10_90 (AD: $n=183$; GD: $n=82$) polymorphism within the CXCL10 gene. Additionally patients with Graves' disease and healthy controls from Poland (GD: $n=181$; HC: $n=147$) and Serbia (GD: $n=177$; HC: $n=151$) were genotyped for the CXCL10_89 polymorphism using real time PCR.
Results
In patient with Addison's disease, the CXCL10_89 (G/A) heterozygosity was more frequent (58.6 vs 46.7%) while the (G/G) homozygosity rate (25.9 vs 36.5%) was found less than in healthy controls ($P=0.031$). No differences were observed in the genotype-frequencies for CXCL10_90 and CXCL10_89 polymorphisms in Graves' disease.
Conclusion
CXCL10_89 gene polymorphism was significantly associated with Addison's disease in the German population but not with Graves' disease. Our results point out that CXCL10 could be a candidate gene in the pathogenesis of Addison's disease. Nevertheless, functional studies are underway to put these findings into physiological contexts.

P166

The prevalence of transsexualism (TS) and other gender identity disorders (GID) in Russia: an overview of existing data

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Background
The prevalence of TS and other GID, as well as men/women ratio (MWR) of the pathology is the topic of a high interest for professionals in this field. The earlier data from Russia reported the MWR as 1:3 and were different from reported by other countries.
Objective
To study the current prevalence of TS and other GID in Russia.
Materials and methods
We summarized the data collected in 2006 by four Moscow clinics, experienced with GID: Research Center for Endocrinology, Moscow Center for Psychoendocrinology, Russian National Research Center for Surgery, Research Center for Psychiatry. Statistical analysis was done with STATISTICA (StatSoft Inc., USA, version 6.0) software.
Results
In 2006, there were in average 31.6 men and 30.8 women who addressed with the request of sex change for the first time. Among them, diagnosis of TS was ascertained in 16.6 (52.5%) men and in 21.3 (69.2%) women. Another GID were diagnosed in 15 (47.5%) men and 9.5 (30.8%) women. Hence, the MWR in patients with TS formed 1:1.3 and 1.6:1 in patients with other GID, respectively.
Conclusion
The new data are different from reported earlier. This fact could be explained with the lack of accurate differential diagnostics between TS and other GID. The second aspect was that the data were prevalently estimated by surgeons and included not only transsexuals, but all people with different GID who requested for surgery. The third aspect was that the more frequent surgery in transsexuals was mastectomy in female-to-male transsexuals (because vagino- or phalloplasty were more complicated and more expensive), so these patients requested to surgeons more often. Our current data reflect the situation more widely, because now we possess the data not only from surgeons, but also from endocrinologists and physiatrists.

P167

***In vivo* effects of chronic contamination with 137 cesium on testicular and adrenal steroidogenesis**

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More than twenty years after Chernobyl nuclear power plant explosion, radionuclides are still mainly bound to the organic soil layers. Currently, the radiation exposure is dominated by the internal exposure to gamma-radiation following the decay of ^{137}Cs, due to presence of ^{137}Cs into the food chain. Because of this persistence of contamination with ^{137}Cs, questions regarding public health for people living in contaminated areas were raised. Several studies report an increase number of various malfunctions affecting the cardiovascular, nervous and immune systems, in addition to a large increase of thyroid cancers. Moreover, it has been shown that ^{137}Cs accumulate in different organs such as the endocrine glands, the heart and the spleen.

Up to now the effects of the radionuclides such as ^{137}Cs have been poorly investigated on the testicular or adrenal steroidogenesis. Studies on 'liquidators' show some modifications of sperm parameters, along with perturbations within the levels of cortisol, ACTH, and testosterone have been observed following ^{137}Cs irradiation, but the effects of chronic internal contamination has not been studied yet. We investigated the biological effects of chronic exposure to ^{137}Cs on testicular and adrenal steroidogenesis metabolisms in rat. Animals were exposed to radionuclide in their drinking water for 9 months at a dose of 6500 Bq/l (610 Bq/kg per day), a dose that can be found in contaminated areas near Chernobyl.

Cesium 137 contamination decreases the level of circulating 17β-estradiol, and increases corticosterone level. In testis, several nuclear receptors messenger expression is disrupted; levels of mRNA encoding LXRα alpha and LXR beta are increased, whereas FXR mRNA presents a lower level. Adrenal metabolism presents a paradoxical decrease in *cyp11a1* gene expression. In conclusion, our results show for the first time molecular and hormonal modifications in testicular and adrenal steroidogenic metabolism, induced by chronic contamination with low doses of ^{137}Cs.

P168

Contamination with depleted or enriched uranium differently affects steroidogenesis metabolism in rat

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Uranium is the heaviest naturally occurring element found in the Earth's crust. It is an alpha-emitter radioactive element that present both radiotoxicant and chemotoxicant properties. Uranium is present in environment as a result of natural deposits and releases by human applications (mill tailings, nuclear industry and military army). Populations could thus be exposed to uranium either through their drinking water or the food chain.

Natural uranium can be radiologically enriched, the by-product of this enrichment is then called depleted uranium. Enriched uranium is three time more radioactive than depleted uranium.

Few studies have been conducted regarding the effects of uranium contamination on reproduction or steroidogenesis. Hormonal levels were modified among uranium miners, whereas no effects were observed in Gulf war veterans with retained fragments of depleted uranium shrapnel.

To distinguish chemical versus radiological effects of uranium on the metabolism of the steroids in the testis, rats were contaminated via their drinking water with depleted or enriched uranium. Animals were exposed to radionuclides for 9 months at a dose of 40 mg/l (560 Bq/l for depleted uranium, 1680 Bq/l for enriched uranium). This dose represent double highest concentration find in some wells of Finland.

While depleted uranium did not seem to significantly affect the production of testicular steroid hormones in rats, enriched uranium significantly increased the level of circulating testosterone. Enriched uranium contamination pointed out significant increases in the mRNA levels of synthesis enzymes, while depleted uranium contamination induces no change in these genes expression. Moreover, expression levels of nuclear receptors, as well as the transcription factors were modified following enriched uranium contamination.

In conclusion, our results show for the first time a differential effect among depleted or enriched uranium contamination on the testicular steroidogenesis. This study didn't show pathological consequences, but raises questions about the of uranium chronic contamination on human being.

P169

Endocrine effects of a coat-color mutation Star in farm-bred silver foxes Vulpes vulpus

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Captive breeding of wild animals is a first step of domestication. The process is accompanied by a number of adaptations to captive conditions, which resulting in behaviour, physiological and morphological changes. One of morphological consequences of domestication is a coat-colour mutation named Star (S) that is characterised by some unpigmented areas on the skin. The dominant mutation was first revealed in farm-bred populations of silver foxes, but the highest frequency of this mutation was observed in silver foxes selected artificially for domestic behavior (nonaggression to human). The aim of the present work was to investigate effects of Star gene on hormonal activity of the ovaries and adrenals, and fertility in adult silver foxes selected for domestic behaviour ($n=38$) and unselected control ($n=37$). At 22–28 days of pregnancy and during midanoestrus, the homozygous (SS), heterozygous (Ss) and wild-type (ss) females from selected and unselected populations were euthanized, blood samples were taken, gonads and adrenals were dissected. Plasma levels of progesterone, oestradiol and cortisol, as well as gonadal content of progesterone and oestradiol, and adrenal content of cortisol and progesterone were measured by RIA. Fertility was estimated by the number of corpora lutea and implantation sites in the same groups. The Star allele decreased plasma concentrations of progesterone and cortisol, ovarian progesterone contents and adrenal contents of both hormones during pregnancy with a most pronounced effect in selected groups. We also showed negative effects of this gene on the number of corpora lutea and implantation sites in selected groups, and on ovarian and adrenal weights in both behavioural groups. In conclusion, our results indicate that in addition to its effect on pigmentation, the S allele also causes a number of endocrine pleiotropic effects, which are more expressed in animals selected for domestic behaviour.

P170

Serum levels of the soluble isoform of the receptor of advanced glycosylated end products (sRAGE) are increased in women with PCOS

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Serum levels of soluble RAGE (sRAGE total), the soluble isoform of the receptor of advanced glycated end products (AGEs) that lacks the transmembrane and intracytoplasmic domains of RAGE, has been found elevated in type 2 diabetics and non diabetic subjects and positively correlated with AGEs levels. In women with PCOS, while serum AGEs have been demonstrated to be elevated, sRAGE levels have not been determined as yet.

The aim of the present study was to determine sRAGE serum levels in PCOS women compared with the levels in healthy controls and investigate the potential associations of sRAGE with metabolic and endocrine parameters in PCOS women. The study was approved by the local ethical committee.

Fifty-one non diabetic reproductive-aged women with PCOS (defined by Rotterdam criteria), receiving no medical treatment and 10 controls were studied. sRAGE, AGEs, androgen, estradiol serum levels and homeostatic assessment model (HOMA) were determined. Women with PCOS compared with controls of similar age and body mass index (BMI) had significantly increased sRAGE levels (677.1 ± 51.57 vs 475.6 ± 22.69 pg/ml, $P=0.019$), which were correlated positively with AGEs levels ($r=0.533$, $P=0.009$). sRAGE levels did not correlate with BMI, androgen levels and HOMA.

These data demonstrate, for the first time, that non diabetic PCOS women have increased levels of sRAGE, which correlate positively with AGEs levels, but are independent of obesity, hyperandrogenemia and insulin resistance. The role of elevated serum sRAGE levels, in PCOS remains to be explored.

P171

Effects of dietary advanced glycation end products on endocrine and metabolic parameters of women with polycystic ovarian syndrome

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In female rats, diet enriched in advanced glycation end products (AGEs) has been associated with increased serum testosterone levels and deposition of dietary glycotoxins in ovarian tissue.
Women with PCOS present increased serum AGE levels, which are acutely elevated after intake of a single meal high in AGE content. In this study the effects of a hypocaloric diet and an AGE-enriched hypocaloric diet were investigated, on the endocrine and metabolic profile of PCOS women.
Eleven women with PCOS, defined by Rotterdam criteria, were assigned for two months to a hypocaloric regular diet followed by two months of a hypocaloric AGE-enriched diet. At the end of each period endocrine parameters were determined.
PCOS women on hypocaloric diet showed a significant reduction on BMI ($P=0.0276$), which was followed by a significant reduction on HOMA ($P=0.0035$), but not significant changes on AGEs ($P=0.6073$) or Testosterone concentrations ($P=0.7857$). In post hypocaloric-AGE-enriched diet, without significant changes in BMI ($P=0.29$) and HOMA ($P=0.1560$), testosterone levels ($P=0.0007$) were increased in comparison to their status during hypocaloric diet and to baseline. Additionally, the difference of AGEs levels from hypocaloric diet to high AGEs diet were significantly higher ($P=0.0312$).
Increased dietary intake of AGEs in hypocaloric diet is associated with significant increases in androgen levels, contributing to abnormal hormonal profile in women with PCOS. Since in the ovarian compartments from polycystic ovarian tissue the AGE and their receptor RAGE have been determined immunochemically, the role of dietary AGEs in PCOS needs to be explored.

Diabetes and cardiovascular diseases

P172

Changes in the sympathetic and sensory innervation of the tail artery, adrenal gland and male reproductive organs of glucose-intolerant Goto-Kakazaki (GK) rats

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Twelve-month-old GK rats that showed glucose intolerance were compared with age matched Wistar rats. The concentrations of noradrenaline (NA), adrenaline (ADR) dopamine (DOP), Neuropeptide Y (NPY), and calcitonin gene-related peptide (CGRP) were measured in tissue samples. The objective was to assess whether glucose intolerance was associated with changes in the noradrenergic and peptidergic nerves innervating the tail artery (TA), seminal vesicle (SV) and corpus cavernosum (CC), and the adrenal gland (AG). The tail artery was divided into three segments – proximal, middle and distal – of approximately 5 cm in length, because these contained the nerve endings of some of the longest nerves in the body of the rat.
Tail artery
In the tail artery, (NA) was significantly increased in the distal segments but was reduced proximally. (ADR) was unchanged proximally, but increased significantly in the middle and distal segments. (DOP) was always significantly less than the control levels, but increased from proximal to distal segments. (NPY) was significantly increased in the proximal and distal segments, and (CGRP) was significantly increased throughout the length of the artery, particularly in the proximal segment.
Seminal vesicle, corpus cavernosum and adrenal gland
(NA) increased significantly in the CC, but was reduced in the SV and AG. (ADR) was significantly reduced in the SV and AG, but unaffected in the CC. (DOP) was significantly increased in the ADR and CC, but fell in the SV. (NPY) and (CGRP) did not change in these tissues.
These changes indicate that there are significant changes in the longest peptidergic sensory and sympathetic axons in the 12 month-old GK rat, and the changes in (NA) in the distal tail artery and in the CC are similar to those described in the streptozotocin diabetic rat[1,2].

References
1. Morrison *et al. Mol Cell Biochem* 2004 **261** 77–82.
2. Morrison *et al. Int J Impot Res* 2007 **19** 509–516.

P173

Anxieties of type one diabetic patients

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Background
Diabetes mellitus type 1 leads to various complications which make diabetic patients anxious. In this study, anxieties of 11 diabetic patients who could take their blood sugar under control were investigated and solutions were suggested and done.
Patients and methods
Eleven patients with diabetes mellitus type 1 listed their anxieties and ranked them in matris. The most important anxiety of these patients was blood sugar level changes after each meal. The practical and available solution for decreasing this anxiety was awareness of blood sugar level after meals. This solution was suggested by the patients themselves. For this purpose, the amount of calorie of meals and blood sugar level after 30 to 60 min after each meal were measured by the patients for 1 month.
Results
Interventions resulted in decrease of anxiety about blood sugar changes after meals. In the end, nine patients declared that their anxiety was decreased.
Conclusion
Awareness and knowledge are the main factors for decreasing anxieties and participation of diabetic patients in solving their problems can improve their lifestyle.

P174

Opportunistic screening for type 2 diabetes mellitus in the out-patient clinic: experience from Basrah, Southern Iraq

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Background
Opportunistic screening is more efficient than population screening for diabetes mellitus. The aim is to describe the prevalence of unrecognized diabetes mellitus (DM) among out-patients clinic attending in a major hospital in Basrah, Southern Iraq.
Methods
A cross-sectional observational study. The study conducted for the period between January 2006 to end of July 2007. Patients, who attended the out-patient clinic in Al-Faiha hospital in Basrah, were enrolled in this study if they were neither known diabetics nor had florid features of DM, and their age is 18 and above. New DM diagnosis was based on FPG equal to or more than 126 mg/dl (7.0 mmol/l) on two occasions.
Results
The total study sample was 15 505, of whom 7983 (51.5%) were men and 7522 (48.5%) were women. Age range was 44.83 ± 15.83. New DM was seen in 1036 (6.7%). Mean age was higher among those with new diabetes 52.51 ± 12.12 vs 44.29 ± 15.92 (P-value <0.001). No clear difference was seen between both sexes regarding incidence of DM (0.007). All anthropometric indices value was clearly higher among those with diabetes. At age of 45 and above, there was a clear difference between the diabetic group and non-diabetics (P-value <0.001) with prevalence of DM more among those at age of 45 and above in both sexes.
Conclusion
Using opportunistic screening for DM in major hospital in Basrah, Southern Iraq, we detected 6.7% new patients with diabetes in the screened population.

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Long term *N*-acetylcysteine and L-arginine administration reduces endothelial activation and systolic blood pressure in hypertensive patients with type 2 diabetes mellitus

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Objective
Reactive oxygen species and nitric oxide (NO) have recently been considered involved in the cardiovascular complications of patients with type 2 diabetes as NO is supposed to loose its physiological beneficial effects, due to the presence of oxygen radicals. For this reason, we tested the effects of L-arginine (ARG) and *N*-acetylcisteine (NAC) administration with the aim to increase NO physiological production reducing free radical formation.
Research design
A double-blind study was performed on 24 male patients with type 2 diabetes and hypertension, divided in 2 groups of 12 patients, which received randomly an oral supplementation of placebo or NAC + ARG (1200 + 1660 mg/die, respectively), for 6 months.
Results
The NAC + ARG treatment caused a reduction of the mean arterial blood pressure, both systolic (143.3 ± 4.0 vs 151.5 ± 3.5 mmHg, $P < 0.05$) and diastolic (84.4 ± 2.0 vs 88.2 ± 0.7 mmHg, $P < 0.05$), total-cholesterol ($P < 0.01$), LDL-cholesterol ($P < 0.005$), oxidized-LDL ($P < 0.05$), hsRCP ($P < 0.05$), ICAM ($P < 0.05$), VCAM ($P < 0.01$), nitrotyrosine ($P < 0.01$), fibrinogen ($P < 0.01$), PAI-1 ($P < 0.05$) and intima-media thickness ($P < 0.02$) during endothelial post-ischemic vasodilation. The HDL-cholesterol level increased ($P < 0.05$). No changes in the others parameters were observed.
Conclusions
The NAC + ARG administration seems to be a potential well-tolerated antiatherogenic therapy since it improves the endothelial function in hypertensive patients with type 2 diabetes by improving NO availability reducing the oxidative stress. Our study's results give prominence to its potential use in the primary and secondary cardiovascular prevention in these patients. Further, clinical studies on a larger scale are needed to support our experimental data.

P176

Adiponectin stimulates monocytic release of CCL2, -3, -4 and -5 concomitantly reduces the surface abundance of CCR1, -2 and -5

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Background
Systemic adiponectin is reduced in patients with obesity and type 2 diabetes mellitus. Peripheral monocytes of type 2 diabetic patients are activated and show a higher surface abundance of CCR2. Inflammatory markers like CCL2 are elevated in the serum of these patients. Therefore, the influence of adiponectin on the release of CC chemokines and the abundance of the corresponding receptors were investigated in monocytic cells.
Methods
Primary human monocytes were isolated and incubated with recombinant adiponectin (10 μg/ml) for 24 h. GeneChip analysis was performed and ten out of 23 known CC-chemokines were found expressed in human blood monocytes by GeneChip analysis and the mRNA of eight of these chemokines was induced by adiponectin.
Results
Elevated CCL2 to CCL5 mRNA and protein secretion were confirmed by real-time RT-PCR and ELISA, respectively. The p38 MAPK inhibitor SB 203580 abrogated adiponectin-mediated release of CCL2 and CCL3. CCL2 binds to the G-protein coupled receptors CCR2, CCL3 and CCL5 to CCR1 and CCR5, CCL4 and CCL5 to CCR5. The surface abundance of these receptors was significantly reduced in adiponectin treated monocytes. The mRNA expression of CCR1 and CCR2 was not affected whereas CCR5 mRNA was slightly elevated. CCR1 protein was lower in adiponectin-treated monocytes and CCR5 was increased when total cell lysates were investigated by immunoblot. CCL2 had been described to induce the mRNA expression of CCR2 and TGF-beta? in human monocytes. However, the abundance of these mRNAs was not altered in adiponectin-stimulated monocytes arguing against an autocrine or paracrine effects of this chemokine.
Conclusions
Therefore, it is concluded that adiponectin induces the release of CCL2 to CCL5 and simultaneously reduces the surface expression of the corresponding receptors and thereby may prevent an autocrine or paracrine activation of monocytes as has been demonstrated for CCL2 herein.

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Effects of the omega-3 polyunsaturated fatty acids in the treatment of cardiovascular autonomic neuropathy in Type 2 diabetes mellitus patients

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Background and aims
The aim of this study was to assess the effect of docosahexaenoic (DHA) and eicosapentaenoic acid (EPA) on the heart rate variability (HRV), dynamics of such biochemical parameters in patients (pts) with Type 2 DM and cardiovascular aunonomic neuropathy (CAN).
Materials and methods
Forty-nine pts with CAN (54 ± 5 years) were allocated in two groups. Pts of group A ($n = 17$) were receiving capsules of fish oil every day ('Epadol': 2.0 g EPA, 2.0 g DHA and 0.1% α-tocopherol acetate); B ($n = 14$) – capsules of olive oil. The duration of the study was 3 months. We investigated the protein-kinase C (PK-C), Na^+, K^+-ATPase, Ca^{2+}, Mg^{2+}- ATPase activities, RBC's membranes fatty acids composition, ^{125}I-6-ketoprostaglandin $F_{1\alpha}$ (6-ketoPGF$_{1\alpha}$) and ^{125}I-thromboxane B_2 (TXB_2) levels in the blood plasma, platelet aggregation parameters.
Statistics
ANOVA.
Results
It has been discovered that manifestation of the CAN is accompanied by decrease of the Na^+, K^+-ATPase, Ca^{2+}, Mg^{2+}-ATPase activities ($P < 0.001$), 6-ketoPGF$_{1\alpha}$, EPA level, EPA/arachidonic acid ratio with simultaneously increase of TXB_2 level, PK-C activity, QTc interval parameters. Analysis of aggregatory curves shows that platelets in Type 2 DM with CAN began to aggregate earlier and the speed (0.75 ± 0.02 U/min, $P < 0.001$), stage of aggregation (27.78 ± 1.15 MU/min, $P < 0.01$) increase. After 3 months of treatment there was a decrease of TXB_2 level ($P < 0.001$), activity of PK-C (12.37 ± 4.11 pmol ^{32}P/mg protein per 1 min, $P < 0.001$), degree and speed of an aggregate of thrombocytes with simultaneous increase of EPA, 6-ketoPGF$_{1\alpha}$ levels, EPA/arachidonic acid ratio, Na^+, K^+-ATPase, Ca^{2+}, Mg^{2+}-ATPase activities in the group A were marked. Also, we observed significant improvement of cardiovascular autonomic tests, HRV parameters, decrease of QTc interval ($P < 0.01$).
Conclusions
DHA and EPA may exert cardioprotective, antithrombic effects and may be used for effective treatment of diabetic CAN.

P178

Omega-3 polyunsaturated fatty acids and simvastatin in the treatment of type 2 diabetic patients with cardiomyopathy

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Background and aims
The aim of this study was to assess the effects of simvastatin (SIM), eicosapentaenoic (EPA), docosahexaenoic acid (DHA) on dynamics of such biochemical parameters in type 2 diabetic patients with cardiomyopathy (DCMP).
Materials and methods
Twenty-five patients with DM and DCMP received SIM 20 mg tid (group A); B ($n = 37$) – capsules of fish oil ('Epadol': 2.0 g EPA, 2.0 g DHA and 0.1% α-tocopherol acetate), C ($n = 24$) – SIM 10 mg plus 'Epadol'. All patients were on the same diet. We investigated the lipid profile, ^{125}I-6-ketoprostaglandin $F_{1\alpha}$ (6-ketoPGF$_{1\alpha}$), ^{125}I-thromboxane B_2 (TXB_2) concentrations, liver enzymes activities in the blood plasma; Na^+, K^+-ATPase activities, fatty acids level in the membranes of RBC's. The duration of the study was 3 months.
Statistics
ANOVA.
Results
Lipid disorders (high level of total cholesterol (TC), LDL-C, triglycerides (TG, 7.3 ± 10.1; 4.5 ± 0.3; 2.87 ± 0.2 mmol/l, $P < 0.05$)) and decrease level of HDL-C

in the patients with DCMP are accompanied by a decrease of the Na^+, K^+-ATPase activities in the RBC's membranes. There is a considerable increase in the TXB_2 level and a decrease in serum 6-ketoPGF$_{1\alpha}$, EPA, DHA levels in the RBC's membranes. It has been discovered that the monotherapy by SIM is accompanied by negative dynamics of liver enzymes activity. After 3 months of treatment there was a more significant decrease in LDL-C, TC, TG concentration, TXB_2 level (152.5 ± 16.9 pg/ml, $P<0.001$) with simultaneous increases of EPA level, EPA/arachidonic acid ratio, Na^+, K^+-ATPase (0.04 ± 0.003 vs 0.09 ± 0.004 mmol P_i/mg protein per 1 h, $P<0.001$) and the concentration of 6-ketoPGF$_{1\alpha}$ in the 3rd group ($P<0.001$).
Conclusion
The combined purpose SIM and 'Epadol' significantly improve the lipid profile, state of prostacyclin I_2-thromboxane A_2 system, lower a doze SIM, that allows to recommend their combination in the rational-proved treatment of patients with DCMP.

P179

Degree of control of cardiovascular risk factors (CRFs) in patients with type 2 diabetes mellitus (T2DM) stratified by diabetes therapy

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Control of CRFs in T2DM patients results in a clinically important reduction in the risk of death and complications related to diabetes.
Objective
To assess the degree of control of modifiable CRFs in treated T2DM patients, stratified by diabetes therapy.
Materials and methods
Cross-sectional study in 574 T2DM patients who attended our clinic for a routine follow-up. Fasting plasma glucose (FPG), HbA1C, HDL-Cholesterol (HDL-Ch), LDL-Ch and triglycerides (TGs), systolic (SBP) and diastolic blood pressure (DBP), BMI, waist circumference (WC), smoking status and cardioprotective medications were extracted from over diet dose. We applied the ADA recommendations for our comparisons. The use of cardioprotective medications was also evaluated.
Results
Mean (±S.D.) age was 67.2 ± 11.4 years. Overall, 43% patients received insulin and 57% took taking oral hypoglycaemic drugs (OHD). Insulin users tended to be older, to have longer duration of diabetes, higher WC, and higher prevalence of obesity, abdominal obesity, metabolic syndrome (MS), coronary artery disease (CAD) and hypertension, than subjects taking OHD. Moreover, had higher BMI ($P=0.004$), SBP ($P=0.002$), FPG ($P=0.000$), and HbA1C ($P=0.000$), than patients taking OHD. The percentage of subjects who reached the HbA1C and SBP target and the number of male patients who achieved the WC target was higher among subjects taking OHD than in those on insulin ($P=0.000$, 0.03, 0.016 respectively). More subjects on insulin than on OHD were taking antiplatelet therapy ($P=0.001$), ACEIs/ARBs ($P=0.004$), beta-blockers ($P=0.0035$), calcium channel blockers ($P=0.0031$) and diuretics ($P=0.01$).
Conclusions
The higher prevalence of obesity, abdominal obesity, hypertension, CAD and MS, and the poor control of the majority of CVRFs observed in our diabetic population treated with insulin, supports the need for more aggressive arrangement of their CVRFs.

P180

Determination of asymptomatic interacranial stenotic arteries in diabetic and hypertensive old patients by transcranial doppler sonography

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Background
Ischemic stroke is a major complication of diabetes mellitus. This survey was design to detect asymptomatic intracranial stenosis and determine it's risk factors in diabetic patients older than 50 years with hypertension by transcranial doppler sonography (TCD).
Methods
Between June 2003 and December 2004 all diabetic patients older than 50 years with hypertension and no previous history of cerebrovascular problems, were selected. After completion a questionnaire, evaluation of peak systolic flow velocity (PSV) in middle cerebral (MCA) internal carotid (ICA) and vertebral arteries (VA) was done by TCD. PSV > 120 cm/s for MCA and ICA and PSV > 100 cm/s for VA was defined as significant stenosis. It is approved by local Ethical Committee.
Results
One hundred and eight patient (51 female, 57 male) aged 62.51 ± 7.90 (50–85) years were invited. About 22 (20.3%) patients (11 male, 11 female) had stenosis, 34 (31.4%) stenotic arteries were also determined (8 stenosis in ICA, 8 in VA, 18 in MCA) mean age of 22 patients with and 86 without stenosis were 62.09 ± 6.71 and 62.50 ± 8.23 years respectively, P=NS. Mean duration of hypertension between these two groups were 5.36 ± 8.00 and 3.07 ± 3.39 years respectively P=NS, mean duration of diabetes was 14.09 ± 8.42 and 8.32 ± 6.90 years respectively $P<0.01$. Nine out of 22 (41%) had hyperlipidemia and 6 of them had multiple involvement, but only 1 out of 13 patients without hyperlipidemia had multiple stenosis.
Conclusion
More than 20% of our patients had significant stenosis. MCA was the most common involved artery. There wasn't any relationship between occurrence of stenosis with age, sex and duration of hypertension but a significant correlation was observed between prevalence and severity of stenosis with duration of diabetes and also hyperlipidemia. Periodic evaluation of old diabetic patients is recommended.

P181

The impact of family history of type 2 diabetes mellitus on insulin sensitivity in lean subjects with polycystic ovary syndrome

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Objective
Polycystic ovary syndrome (PCOS) is a common endocrine disorder, characterized by hyperandrogenism and chronic anovulation. Hyperinsulinemia and insulin resistance are well-documented features of the disease. In obese PCOS patients, family history of type 2 diabetes mellitus (FH-DM) is shown to be related with a decrease in insulin sensitivity, however not much is known about lean PCOS cases. The aim of this study was to determine the relationship between FH-DM and insulin resistance in lean PCOS subjects.
Methods
Nineteen lean PCOS patients (mean body mass index-BMI: 23.06 ± 2.71 kg/m^2) were recruited into the study. Subjects were separated into two groups regarding their FH-DM status. Mean age, BMI and metabolic syndrome parameters were similar between the FH-DM negative ($n=10$) and FH-DM positive ($n=9$) groups. Insulin sensitivity was evaluated by using the homeostasis model assessment of insulin resistance; HOMA-IR formula and the hyperinsulinemic euglycemic clamp method from which insulin sensitivity was derived from glucose disposal rate expressed as mg/kg per min and indicated as 'M' index.
Results
Mean M value of the whole group was 5.97 ± 1.58. Mean HOMA-IR and M values of the FH-DM positive and FH-DM negative groups exhibited insignificant difference; 2.02 ± 0.86 vs 1.98 ± 1.11 and 5.41 ± 1.20 vs 6.48 ± 1.77, respectively ($P>0.05$).
Conclusion
In this study, we found insignificant difference regarding insulin sensitivity markers among lean PCOS cases with and without a family history of type 2 diabetes mellitus. We propose that a positive FH-DM had no impact on insulin sensitivity in lean PCOS subjects.

P182

Rapid acting insulin analogues are superior to normal human insulin in intensified insulin treatment of type 2 diabetes mellitus

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Postprandial hyperglycaemia is a cardiovascular risk factor in diabetic patients. Therefore, younger type 2 diabetic patients are best treated by intensified insulin therapy (IIT) for effective control of postprandial hyperglycaemia. It is unclear, however, whether rapid acting insulin analogues were superior to normal human insulin when used in IIT by patients with type 2 diabetes mellitus.

We carried out a randomised open intra-individual cross-over trial to compare blood glucose (BG) responsiveness to insulin analogues (preprandial versus postprandial injections of insulin lispro, aspart or glulisine) with that to normal human insulin (injected 30 min versus immediately before meal). BG was measured before and one hour after the three main meals and at bedtime. Seventy insulin-naïve type 2 diabetic patients (age, 62±2 years (mean±S.E.M.), known duration of diabetes, 7±2 years, BMI, 30.3±1.1 kg/m^2) participated at this study.
Whereas, BG measured one hour after meal did not differ after preprandial versus postprandial injections of rapid acting insulin analogues, postprandial BG was lower after injections of normal human insulin 30 min versus immediately before meal (171±6 vs 167±5 mg/dl and 155±6 vs 187±7 mg/dl, respectively, $P<0.05$ (ANOVA)). The averages of the 7-point BG profiles were similarly different: 145±5 vs 142±4 mg/dl and 138±3 vs 154±4 mg/dl, respectively, $P<0.05$). Prandial insulin requirement was lower with insulin analogues (24±2 vs 30±2 IU/day, $P<0.05$). There was no hypoglycaemic event throughout the study.
Whereas in IIT normal human insulin should be injected 30 min before meal, both preprandial and postprandial injections of insulin analogues can be allowed. Since patients benefit from injecting prandial insulin immediately before or even after meal and since less insulin is required with rapid acting insulin analogues, insulin analogues are superior to normal human insulin in type 2 diabetes mellitus.

P183

Melatonin–insulin relationships in rats and humans

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There is a well documented link between melatonin and insulin. Morphological, molecular and functional investigations have shown that the pineal hormone melatonin (MT) influences the insulin secretion. The effects were mediated by specific, high-affinity, pertussis-toxin-sensitive, G-protein-coupled MT1- as well as MT2-membrane receptors which were detected in pancreatic tissue and islets of rats and humans and additionally in rat insulinoma cells INS1. Using the Gi-protein-adenylylcyclase-cAMP- and possibly the cGMP-pathway, MT decreases the insulin secretion, whereas using the Gq-phospholipase C-IP3-pathway MT increases the insulin secretion.
For further analysis of MT-insulin-interactions, plasma MT levels were measured in diabetic rats and humans. In this context, own recent investigations have proven that type 2-diabetic rats and humans displayed a decreased MT level whereas type 1-diabetic rats (induced by streptozotocin, STZ) showed an increased plasma MT level. In addition, the rate-limiting enzyme of the MT synthesis, the arylalkylamine-*N*-acetyltransferase (AANAT), was investigated by quantitative RT-PCR analysis. Furthermore, plasma catecholamines by HPLC technique, pineal insulin-receptor and adrenoceptor status in addition to clock gene transcripts (*Per1* and *Bmal1*) and the clock output gene *dbp* were analyzed in a circadian series by RT-PCR. In conclusion, the results emphasize that increased insulin levels, which were observed in some forms of type 2-diabetes, are combined with a decreased MT level. On the other hand, decreased insulin levels of STZ-induced type 1-diabetes are combined with a higher MT plasma level. Astonishingly, the drastic metabolic disturbances of diurnal rhythmicities of the parameters investigated were maintained. The conserved melatonin rhythm combined with an unchanged day-night-rhythmicity of AANAT reflect that the master clock, the hypothalamic nucleus suprachiasmaticus, was not affected.

P184

Effects of micronutrients supplementation on lipid peroxidation in Type 2 diabetes

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Objective
The present study designed to assess the effect of Mg+Zn, vitamin C+E, and combination of these micronutrients on glycemic control, lipid profile and lipid peroxidation in type 2 diabetic patients.

Materials and methods
In a randomized, double-blind, placebo controlled clinical trial, 69 type 2 diabetic patients were randomly divided into four groups, each group receiving one of the following daily supplement for 3 months; group M: 200 mg magnesium and 30 mg zinc ($n=16$), group V: 200 mg vitamin C and 150 mg vitamin E ($n=18$), group MV: minerals plus vitamins ($n=17$), group P: placebo ($n=18$).
Results
Results indicate that after 3 months of supplementation mean serum levels of HDL-C and apolipoprotein A1 increased significantly in the MV group by 24% (50.4±19.3 vs 40.6±10.8 mg/dl) and 9% (170±34 vs 156±24 mg/dl), respectively ($P<0.01$). Levels of fasting serum glucose decreased in MV group ($P<0.05$). Malondialdehyde concentrations decreased significantly in the M and MV groups ($P<0.05$ and $P<0.01$, respectively). Arylesterase activity increased significantly in the M group (91±24 vs 87±24, $P<0.05$) There were no significant changes in the levels of these parameters in the other groups. HbA1, fructosamine, insulin and HOMA score and serum levels of total cholesterol, LDL-C, triglyceride, apolipoprotein B and paraoxonase activities were not altered after supplementation in all 4 groups.
Conclusion
It is concluded that since co-supplementation of Mg, Zn, vitamin C and E significantly increases HDL-C and apolipoprotein A1 and improves fasting serum glucose and lipid proxidation, supplementation of these micronutrients should be recommended for the type 2 diabetic patients based on their daily requirements.

P185

Metabolic improvement in diabetic patients under education before implantation of the insulin sibcutaneous continuous infusion (ISCI)

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Introduction
ISCI treatment is useful to optimize treatment in patients with DM1. Before implantation, is necessary the education of patients, as in technical aspects so in the handling of diabetes with this therapy.
Objective
Evaluate the results of the diabetological education previous to the implantation of the ISCI and the repercussion of this program in patients metabolic control.
Material and method
Forty-seven diabetic patients with indication of insulin pump were educated. They were distributed in 4–5 people groups. Age: 31.7 years ±12.45 (12–64). Fifty-six percent women and 44% men. Evolution of diabetes: 16.2 years ±10 (4–40). Indication of pump determined by bad metabolic control in 21 patients (44.6%), hypoglycemia in 15 (31.7%), gestation in 7 (14.8%) and glycemic instability in 4 (8.5%). It was organized in classes of 1.5 h, 1 or 2 afternoons weekly, with theoretical and practical contents. The program consisted of 6 classes: approach to the therapy (indications, advantages and disadvantages), basic concepts (factor of sensitivity, ratio, basal line and bolus), feeding by CH countings, daily and pump life (deports, disconnection, trips, hour change), acute complications and technical handling of the pump.
Results
Once completed program, the average HbA1c descended respect to 2 months previous one (−0.67%), being higher when in the group in whom the pump was indicated by bad metabolic control (1.12%).

HbA1c (%)	Total ($n=47$)	Bad metabolic control ($n=21$)	Glycemic inest-ability ($n=4$)	Pregnancy ($n=7$)	Hypoglycemias ($n=15$)
Pre	8.48±2.49	10.12±2.74	7.75±0.70	7.58±1.60	6.79±0.86
Post	7.81±1.99	9.00±2.09	7.40±0.52	7.10±2.07	6.58±0.97
P	<0.001	<0.001	0.10	0.19	0.12

Conclusions
In candidates to pump by bad control, a program of diabetological education served to improve this control before initiate this therapy. The educative programs make an improvement in the metabolic control and a motivation in the self-care of the patient. Its application to all the diabetics would be advisable.

P186

Comparison of patient's life quality between inhalated insulin therapy (Exubera) and conventional treatment

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Objectives
To evaluate satisfaction with treatment in diabetic patients under therapy with inhaled insulin (exubera).
Material and methods
Thirty-seven diabetic patients were evaluated, 27DM1 (75%) and 9DM2 (25%) that initiated treatment with Insulin inhaled (EXUBERA) between August and October 2007. Twenty-five men (69.4%) and 11 women (30.56%), between 18 and 71 years old (38.4±16.4) and with a time of evolution of diabetes between 0 and 47 years (13.9±9.9). They presented an HbA1c average: 8.13±2.1%. They filled the DTQ1s before beginning the treatment (group 1/G1) and 1 month later (group 2/G2). It was made a non-parametric test to match the results (Wilcoxon).
Results
Eight aspects were valuated that patients had to score in a scale between 0 and 6. Satisfaction with present treatment: G1 3.50±1.1, G2 5.83±3.8, $P<0.001$. Frequency of hyperglycemias G1 3.02±1.8, G2 1.80±1.5, $P<0.01$. Frequency of hypoglycemias G1 1.6±1.6, G2 1.27±1.6 non significance. Convenience of treatment G1 4.44±1.5, G2 5.77±0.4, $P<0.001$. Flexibility of treatment G1 4.33±1.6, G2 5.19±0.9, $P<0.001$. Knowledge of diabetes G1 4.58±1.5, G2 5.52±0.6, $P<0.001$. Recommend treatment to other G1 3.72±1.4, G2 6.0±0.0, $P<0.001$. Disposition of continuing treatment G1 2.22±1.8, G2 6.0±0.0, $P<0.001$.
Conclusions
Diabetic patients showed more satisfaction with the inhalated insulin therapy than with their previous treatment. There were no significant differences in the perception of hypoglycemias but they did appreciate less episodes of hyperglycemias. The greater satisfaction with inhaled insulin therapy is shown by the disposition to continue the treatment and to recommend it to other patients.

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Satisfaction degree and metabolic control in patients with glucose continuous monitoring system

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Objective
To study the improvement of metabolic control and patients satisfaction with glucose continuous monitoring systems (Guardian real time).
Material and method
Fifteen DM1 patients studied (age: 33.8±13.40, years of evolution: 17.06±11.32) in intensive treatment (5 multidoses, 10 insulin pump) which were made one 4 days long blind monitoring Period 1 (blind period) with sensor CGMS Gold. Next another monitoring was made with a real time system with alarms of hyperglycemia and hypoglycemia, Guardian RT, Period 2 (real time period). These 2 monitorings were consecutive in each patient, establishing in the second period the levels of alarm of hypoglycemia in 50 mg/dl and hyperglycemia in 200 mg/dl. We studied in both periods: average glycemia, glycemia variability, percentage of time in hyperglycemia (>180), normal glycemia and hypoglycemia (<70). A satisfaction questionnaire about the systems of continuous monitoring was made.
Results

	Period 1 (blind)	Period 2 (R.T)	*P*
Average glyc	157.17±34.30 (110–224)	137.49±21.99 (115–203)	0.053
Variability	73.72±19.60 (38–103)	48.23±13.20 (25–75)	<0.005
% High	32.56±19.25 (7.0–75.0)	19.90±13.87 (1.23–56.03)	<0.05
% Euglycemia	55.49±17.79 (25.0–86.0)	74.98±14.22 (43.97–98.77)	<0.005
% Low	11.94±8.04 (0.00–29.00)	5.10±5.16 (0–17.00)	<0.005

	Guardian	*P*	CGSM
Satisfaction	4.2	NS	3.8
Recommendable system	5.1	<0.,05	4.1
Uncomfortability	3.8	NS	3.1
Anxiety	1.8	NS	1.9
Interferences with			
Job	1.3	<0.05	2.5
Social life	1.1	<0.05	2.1
Physical activity	2.7	NS	3.1
Sexual life	2.5	<0.05	3.5
Sleep quality	1.5	NS	2.3

Conclusions
We observed during the monitoring in real time:
– Longer time in normal glycemia with less frequency of hypoglycemia and hyperglycemia.
– Smaller glycemia variability.
The monitoring in real time could be useful tool at the time of assuring a better metabolic control and to diminish the exhibition to hypoglycemias.
GURADIAN RT did not generate greater anxiety than the GCMS interfering to a lesser extent in the daily life of our patients.

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Metabolic control and quality of life in patients with DM1 and insulin pump therapy

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Objectives
To value the quality of life improvement and metabolic control in patients with DM1 in the 2 first years of treatment with insulin pump therapy.
Material and methods
We studied 33 DM1 patients, 16 men (48.5%) and 17 women (51.5%), with average age of 34.2±12.2 and 16.3±10.21 years of disease. Pump indication was hypoglycemia in 10 (30.3%), badly metabolic control in 14 (42.4%), pregnancy in 5 (15.2%) and glycemic inestability in 4 (12.1%). Following parameters pre-treatment were valued to the 3, 12 and 18 months: HbA1c, total insulin (IU/kg), BMI, glycemia average, hypoglycemias and number of controls/day. All filled the WHO questionnaire of treatment satisfaction previous to implantation of I.S.C.I., to 6 and 12 months.
Results

	Pre	Beginning	6 m	12 m	18 m
HbA1c	8.0±1.9	7.5±1.6†	6.8±1.3†	6.4±0.5*	6.3±0.7
IU/kg	0.76±0.3	0.56±0.3†	0.57±0.2†	0.65±0.3	0.45±0.1
BMI	25.4±4.3	25.05±4.0	25.37±3.9	25.14±3.2	25.89±2.6
Glycemia	157.2±32	155.4±24.5	151.7±50.1	156.5±52.1	151.4±12.3
Variability	86.7±20.3	83.2±20.7	73.3±23.8*	96.5±45.2	79.1±15.1
Hypoglycemias/month	8.1±6.5	10.6±8.2	4.5±3.9*	6.08±5.2*	4.20±2.4
Controls/day	1.76±0.8	3.59±1.9	4.14±1.6	3.2±1.2	3.46±0.5

*$P<0.05$; †$P<0.01$.

Conclusions
A significant reduction of HbA1c was obtained in patients with insulin pump, specially during the first month. The HbA1c descended before treatment in relation to the educative program. The number of hypoglycemias decreased three months after the beginning. A greater satisfaction with I.S.C.I was observed at the 6 and 12 months.

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Utility of ambulatory blood pressure monitoring in diabetological consulting

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Objectives
To check possible advantages of ambulatory blood pressure monitoring (ABPM) in diagnosis and disease control respect to blood pressure conventional measurement ways.
Also to study the circadian rhythm of each patient to detect higher cardiovascular risk situations.
Material and methods
One hundred and nine patients were included in this study: 44% male, 56% female. ABPM were indicated in four different situations: hypertension diagnosis (45%), treatment efficacy (44%), pheocromocytoma (2.8%), miscelanous (8.2%). Daytime blood pressure values and the night decrease were studied to catalog them as deeper, non-deeper or raiser. ABPM values and consulting ones were compared. Finally, we compared non-deeper/raiser frequency between treated patients and people who acceded for diagnosis.
Results
In the hypertension screening group, 17 of 49 were confirmed, from them 25 (51.03%) were deeper and 24 (48.97%) were non-deeper/raiser.
In the treatment efficacy group, 23 of 43 were wrongly controlled. Nine were deeper (15%) and 36 were non-deeper/raiser (75%).
Blood pressure values to consider hypertension were over 135/85 during the day and over 120/70 during night. We considered deeper if blood pressure decreased more then 10%. Non-deeper if these values decreased less tan this value and raiser if there was an increasement of blood pressure.
Forty of the 109 patients included in the study took benefits by initializing or modifying their current treatment.

	Deeper	Non-deeper	Raiser	Total
Screening group				
No hypertension	18	12	2	22 (44.8%)
Hypertension	7	8	2	17 (34.69%)
Treatment efficacy group				
Good control	5	12	3	20 (46.51%)
Bad control	4	14	5	23 (53.48%)

Conclusions
ABPM was useful to:
1. Diagnose hypertension when this is not possible by common ways.
2. Evaluate antihypertensive treatment efficacy and, if necessary, make changes in this treatment.
3. Identify 'non-deeper and raiser' patients, to value possible treatment that could improve cardiovascular risk.

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The knowledge of society about risk factors for cardiovascular diseases like obesity and prevention of it

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Aims
Increasing the nutritional knowledge of society about risk factors for diabetes mellitus like obesity and cardiovascular diseases.
Background
Cardiovascular diseases are one of the most important cause of death between the people in many countries, and obesity is one of the important causes for cardiovascular diseases. So the society's knowledge about risk factors for it like obesity is very important to prevention of cardiovascular diseases.
Methods
The method was the clinical trial on 84 occupied men. The sampling was at random. After selecting the samples, the first application was completed by the samples. Then the samples were divided into two groups (42 control, 42 test). For the test group, we showed an educational video film, and after one month, the second application was completed by the two groups.
Results
In the first application, the two groups didn't have good knowledge about risk factors for cardiovascular diseases like obesity. In the second application, the control group did not obtain good knowledge, but in test group their knowledge increased significantly ($P<0.001$).
The comparison of knowledge between the two groups, showed statistically important difference ($P<0.001$).
Conclusion
This study showed that the society's knowledge about risk factors for cardiovascular diseases like obesity is minimal, and the educational video film had an important effect on increasing the society's knowledge about it.

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Mediterranean-style diet attenuates inflammation and atherosclerosis markers in type 2 diabetic patients

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Type 2 diabetes mellitus (T2DM) is an insulin-resistant state characterized by an increased low-level inflammation and oxidative stress which are probably the initiation of diabetes-related cardiovascular complications. The aim of the study was to evaluate the impact of Mediterranean-style diet on inflammation and atherosclerosis markers in T2DM patients. Twenty-five overweight/obese T2DM patients (age 54.1 ± 1.9 years, diabetes duration 7.8 ± 1.3 years, $HbA_{1c}\geq7.0\%$) were following (within 3 months) isocaloric dietary recommendation on food intake, i.e. rich in olive oil, fish, vegetables and fruits. Insulin resistance (IR) was calculated using Homeostasis Model Assessment (HOMA). The two-tailed *t*-test was applied for statistical analysis. In T2DM patients, starting values of adiponectin, HDL-cholesterol, paraoxonase activity (PON) and erythrocyte reduced glutathione (GSH) were significantly ($P<0.05$–0.001) decreased whereas levels of sICAM, C-reactive protein (CRP), ferritin and insulin were significantly enhanced ($P<0.05$–0.001) compared to healthy subjects. Adiponectin was increased after dietary intervention ($P=0.018$) whereas tumor necrosis factor alpha (TNF-α) and sICAM were decreased ($P=0.004$, $P=0.007$, respectively), serum ferritin was also diminished ($P=0.028$). There were no significant differences in CRP, HbA_{1c}, HOMA-IR before and after the diet. But HDL-cholesterol, PON and GSH levels were enhanced after diet therapy ($P=0.001$, $P=0.03$, $P=0.004$, respectively). Thus, consumption of the Mediterranean-style diet within 3 months by T2DM patients induced geterogenous anti-inflammatory and anti-atherogenic effects against the background of stable glycaemic state. These finding may implicate a potential use of Mediterranean-style diet as effective non-pharmacological mean for aggressive intervention against diabetes-associated cardiovascular risk factors.

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Endothelin-1 is correlated with TGF-β1 but not with glycemic control in normotensive subjects with type 2 diabetes

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Objectives
Endothelial dysfunction contributes to the development of atherosclerosis in diabetic subjects. Endothelin-1 (ET-1) is an endothelium derived vasoconstrictor. It has been shown that ET-1 levels are increased in a variety of situations such as diabetes, hypertension and obesity. Transforming growth factor beta1 (TGF-β1) is an important cytokine for the development of renal injury in type 2 diabetic patients and associated with hyperglycemia and insulin resistance. Cell culture studies showed that TGF-β may upregulate ET-1 production. Our aim was to demonstrate the possible relations between ET-1 and TGF-β1 in type 2 diabetic patients.

Methods
Forty Type 2 diabetic subjects without any micro or macrovascular diabetic complications, hypertension or microalbuminuria were enrolled. The patients were being treated with glimepride for at least 12 weeks. Patients treated with other oral antidiabetics or insulin, anti-hypertensive drugs, anticoagulants or anti-obesity drugs were not included.
Results
The mean values were; A1c: 7.8%, fasting plasma glucose: 167.9 mg/dl, BMI: 27.5 kg/m^2, waist circumference: 91.0 cm, TGF-β1: 29.68 ng/ml and ET-1: 0.33 fmol/ml. ET-1 levels were not associated with glycemic parameters or anthropometric features. ET-1 levels did not differ between males and females. ET-1 levels were found to be correlated with TGF-β1 levels ($r=0.358$ $P<0.05$).
Conclusion
Our results demonstrate that ET-1 expression is associated with TGF-β1 in type 2 diabetic people. Neither glycemic parameters nor anthropometric features were found to effect ET-1 levels in type 2 diabetic people without diabetes related complications. A possible explanation for this finding may be the strict enrolment criteria for the study. Because of the inclusion of patients without diabetes related complications, ET-1 levels might be found to be unrelated with glycemic parameters.

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Use of three dose premixed aspart: clinical correlates

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Objective
Premixed aspart is being used in once, twice and thrice-daily regimes in persons with diabetes. This study was designed as a cross-sectional, observational study to assess the need for three-dose premixed aspart in different subsets of subjects with type 1 and type 2 diabetes attending an endocrine OPD.
Methods
Records of 200 consecutive patients receiving premixed insulin were assessed to determine the frequency of insulin dosage and its clinical correlates.
Results
Of the 200 records studied, 188 were of type 2 diabetes, 4 of gestational diabetes (GDM) and 8 of type 1 diabetes. Single-dose insulin was prescribed to 38 type 2 diabetes subjects; two-dose to 136 type 2, 2 GDM and 2 type 1 diabetes patients; and three-dose regime to 14 type 2, 2 GDM and 6 type 1 diabetes subjects.
Three dose regime was more commonly used, with respect to the average frequency (11%), in men (13/96: 13.5%) in persons with recently diagnosed diabetes (<1 month) (2/8: 25%), persons with weight >100 kg (9/9: 100%) , with renal transplant (1/2: 50%), with foot infection (2/10: 20%), with urinary tract infection (2/11: 19%), and with type 1 diabetes (75%) or GDM (50%).
It was less often used in elderly subjects >60 years (0/48: 0%), in women (7/104: 6.7%), and in persons with associated hypothyroidism (0/22: 0%).

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Relationship between admission blood glucose (ABG) and homocysteine (HC) levels in patients with acute coronary syndromes (ACS)

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Introduction
The admission glucose may be a predictor of survival and is independently associated with infarct size and higher mortality in patients with ACS. Hyperhomocysteinemia in patients with ischaemic heart disease represents a strong predictor of vascular morbidity. The role of glucose increase above normal levels in non-diabetic patients with ACS is not adequately defined. The aim was the assessment of dependence between admission blood glucose and homocysteine concentrations in patients with ACS.
Material and methods
Ninety-two pts (33F; 59 M), aged 34–82 mean 63.5±12.0 years. In whole group, there were 20 (21.7%) pts with previously diagnosed diabetes. In the time of admission into Intensive Care, glucose and homocysteine levels applying chemiluminescence method (IMMULITE, DTC) in the venous blood had been measured.
Results
In the whole group, myocardial infarct was diagnosed in 52 (56.5%) pts and ACS without acute cardiac necrosis in 40 pts. Mean admission glucose and homocysteine levels in diabetic patients group were 163±62.8 (range 86–264) mg% and 13.1±6.5 (range 6.9–34.3; F 13.8±8.3; M 12.6±4.8) μmol/l respectively. Merely in 12 (16.4%) non-diabetics with ACS admission glucose level didn't exceed 100 mg% (group 1). Mean level glucose and HC in these group was 95.7±3.8 mg% and 12.2±2.6 (F10.9±2.5; M 12.9±2.4) μmol/l, respectively. In the other patients without previously diagnosed diabetes: in 27 (40%) subjects randomly measured glycemia at admission ranged from 101 to 130 mg% (mean 112.1±8.1 mg%) and mean HC 13.8±5.8 (F 13.9±4.9; M 13.7±6.3) μmol/l (group 2); in 16 (21.9%) patients between 131 and 160 mg% (mean 139.9±7.2 mg%) and HC 15.6±6.4 (F 16.3±4.9; M 15.4±7.0) μmol/l (group 3); in 18 (24.6%) above 160 mg% (mean 201.6±34.1) and HC 18.0±9.8 μmol/l (F 17.4±12.8; M 18.5±8.0) (group 4). The myocardial infarction was diagnosed in the 25; 51.8; 56.3 and 72.2% patients of studied groups without diabetes and in 70% of diabetics.
Conclusion
The higher levels of ABG have been connected with the higher HC blood levels as well as with the higher percentage of patients with myocardial infarction.

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K121Q PC-1 gene polymorphism is not associated with postpartum type 2 diabetes in gestacional diabetes mellitus patients

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Women suffering gestational diabetes mellitus (GDM) are a risk group for type 2 diabetes. The genetic determinants are not widely known and controversial data exists about association between K121Q PC-1 gene polymorphism and early type 2 diabetes and/or IR.
The aim of the study was to investigate the relationship between K121Q PC- 1 gene polymorphism and the glucose metabolic alterations, IR or metabolic syndrome in GDM women in the postpartum period.
Patients and methods
Fifty-seven women with previous GDM were reclassified by means of oral glucose tolerance test (OGTT) in the early postpartum using diagnostic criteria of NDDG. Anthropometric and biochemical parameters and systolic and diastolic BP were studied. K121Q PC-1 polymorphism was studied by PCR amplification of the gene ENPP1 (exon4), including the c.361 position which represents the place for the K121Q nucleotide changes. SPSS 12.0 v.s. was used as statistical analysis.
Results
The different genotypes prevalence are shown on table. No significant differences in K121Q polymorphism have been found between the group of patients with glucose intolerance and the patients with normal tolerance.

			OGTT	
Allelic frequencies			Normal ($n=34$)	Intolerance ($n=23$)
Genotype			Prevalence %	
Position c.361	Codon 121	AA121		
A/A (no mutation)	AAG	K	22 (65%)	17 (74%)
C/C (homozygosis)	CAG	Q	3 (9%)	1 (4%)
A/C (heterozygosis)	AAG/CAG	K/Q	9 (26%)	5 (22%)

There were not statistical differences in anthropometric and biochemical parameters between patients with and without the presence Q allele.
Conclusion
The results showed that the K121Q PC-1 gene polymorphism is not associated with type 2 diabetes or glucose intolerance in women with previous GDM.

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Improvement of exercise capacity, muscle strength and insulin resistance in elderly patients with heart failure after treatment with long acting testosterone: a randomized clinical trial

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Background
Patients with congestive heart failure (CHF) show muscle mass wasting and decreased testosterone levels. Long-term testosterone supplementation improves walking distance and glucose metabolism of patients CHF. No studies have investigated the integrated effects of testosterone on exercise oxygen uptake muscle strength and glucose metabolism in patients with CHF regardless of the presence of hypogonadism.
Aim
To assess the effect of a 12-week testosterone administration on maximal exercise capacity, muscle strength and insulin resistance in elderly CHF patients.
Methods
Seventy elderly patients with stable CHF, mean age 71 ± 8 years, ejection fraction 34 ± 1%, NYHA class II/III 38/32, were enrolled. Of these, 35 were randomized to receive testosterone therapy (through intramuscular injection every 6 week) and 35 to receive placebo both on top of maximal medical therapy. At baseline and after 12 weeks all patients underwent echocardiogram, cardiopulmonary test, 6-min walking test (6MWT), quadriceps maximal isometric and isokinetic strength.
Results
At baseline 30% of patients had hypogonadism. Peak VO2 (r 0.44; P 0.02) and quadriceps isometric strength (r 0.39; P 0.01) were both positive related to serum testosterone concentration. After three months, peak VO2 (13 ± 4 vs 16 ± 1 P 0.02), VE/VCO2 (33 ± 7 vs 29 ± 5 P 0.01) distance walked at 6MWT (420 ± 45 vs 480 ± 51 P 0.001), significantly improved from baseline in the testosterone group while were unchanged in the control group; HOMA-IR was significantly reduced in the testosterone group (2.6 ± 1.4 vs 1.8 ± 0.8, P 0.002). Maximal quadriceps isometric (130 ± 28 vs 166 ± 32, P 0.04) but not isokinetic strength was significantly increased at three months in the testosterone group. Increase in testosterone levels were significantly related to improvement in peak VO2. No significant changes in left ventricular function were found.
Conclusion
Long-acting testosterone therapy improves exercise capacity, muscle strengthed glucose metabolism in men with moderately severe heart failure. Testosterone benefits seem to be mediated by metabolic and peripheral effects.

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Circulating Reg1-α plasma levels: potential marker of beta cell regeneration?

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Reg and Reg-related genes are members of a multifunctional family. Under physiological conditions Reg protein is not expressed in pancreatic beta cells, although the Reg protein receptor is expressed. When islets are damaged, the expression of Reg gene is increased in the islets of Langherans. In type 1 diabetes (T1D), insulin secretion may be still detectable in some subjects with long-standing disease indicating the existence of a small population of surviving beta cells or continued renewal of beta cells. Beta cell apoptosis has been shown in pancreas of subjects with long standing T1D indicating that continued source of beta cell replenishment may take place. No measurement is available *in vivo* up to now of this phenomenon. The aim of this study was to evaluate circulating levels of Reg1-α in T1D subjects as a potential marker of beta cell regeneration. We investigated serum samples of newly diagnosed T1D (n=31) (mean age 18.8 years ± 7.4), long standing T1D (n=46) (mean age 36.8 years ± 11.8), type 2 diabetes (n=63) (mean age 63.1 years ± 10.4) and in a group of control (n=50) (mean age 32.7 years ± 12.5).
Levels of Reg1-α were measured using an enzyme linked immunoabsorbent assay. A significant difference was detected in Reg1-α circulating levels between long standing T1D and controls (P=0.03), between newly diagnosed and longstanding T1D (P=0.04), between T2D and newly diagnosed T1D (P=0.002) and between T2D and controls (P=0.0001) No correlation were found between Reg1-α levels and typical markers of diabetes (C-peptide, HbA1c and duration of diabetes).
In conclusion, raised circulating levels of Reg1-α are detectable in T1D and T2D indicating the presence of active expression of Reg1-α gene. Further studies are ongoing to understand the significance of this serum marker and whether it can be used to monitor therapies for beta cell regeneration.

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Influence of ethnicity on prevalence of gestational diabetes

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The prevalence of gestational diabetes (GD) continues to increase worldwide, as diabetes mellitus type 2, with the increasing overweight and age of pregnancy. The Caucasian race is the group with a low risk factor in addition to age <25 years and BMI <25. In Italy, the recent immigration of different ethnies with high natality have changed in the last decade the epidemiologic profile of pregnant women. We assessed the prevalence of gestational diabetes in the view of ethnic modification.
From January 2004 to June 2006, we evaluated 2400 mother–newborn pairs. Glucose challenge test (GCT) was performed at 27–28th week of gestation. If the test was positive (>140 mg/dl at 60″) an oral glucose tolerance test (100 g of glucose) for 3 h was performed and Carpenter-Coustan criteria were followed. Seventy-five percent pregnant women were Italian and 25% non Italian. In the last group, only 13% were European women; among extra-European group 37.1% of women came from Asia, 36.5% from South America, 22.2% from Africa and 4% from USA.
GD diagnosed by GCT in 182 cases (7.5%) was confirmed by OGTT in 132 (5.5%). The incidence of GD has been 4.7% in Italian women and 9% in extra-European women showing a significant difference (P<0.001). Only in 7.5% glucose intolerance was associated with weight increase in pregnancy more than 20 kg. Asian women were at the highest risk (15%) also compared to women from South America (11%). Neonatal complications were hypoglycemia (5.8%) and respiratory distress (3.4%).
Immigrant women are at high risk of impaired glucose tolerance with a higher incidence of cesarian sections and complications of their newborns. The ethnic factor is an independent risk factor in developing impaired glucose tolerance.

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24 h glycemia variability correlates with arterial structure alteration in type 1 diabetes

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Objective
Diabetes mellitus is characterized by vascular complications associated with arterial thickening. Although strict glycaemic control is clinically beneficial, limited or no evidence exists as to whether arterial damage is positively related to blood glucose levels and variability.
Methods
Study purpose was to asses the relationship between arterial thickness and 24 h mean and S.D. of blood glucose in type 1 diabetes. Fifteen type 1 diabetic patients aged 39 ± 1 years (means ± S.E.M.) were enrolled; diabetes aged for 18 years (means). Patients were all normotensive (blood pressure, 128 ± 4/75 ± 2 mmHg) and devoided of clinically evident macro and microvascular complications. Average fasting blood glucose was 160 ± 19.26 mg/dl while Hb1Ac was 7.8 ± 0.35%. Twenty-four hours blood glucose was measured over 24 h every 5 min by a glucometer. The S.D. of blood glucose levels over the 24 h was used as an index of blood glucose variability. Arterial stiffness was measured in two manners: 1) carotido-femoral pulse wave velocity (cfPWV) (Complior), 2) arterial diameter changes/pulse pressure (Reneman Formula, CarDist) the former being measured by an echo-tracking device and the later by a tonometer (pulsepen). Common Carotid artery IMT was measured by standard ecocolor Doppler, 3 cm under bifurcation.
Results
CarDist (4.0 ± 0.4, 1/mmHg 10^{-2}), cfPWV (10.1 ± 0.6 m/s) and IMT (0.54 ± 0.02 mm) showed no correlation with glycated hemoglobine. Only CarDist significantly correlated with fasting blood glucose (r=0.57, P<0.02). IMT shows no relationship with mean blood glucose both when assessed in the clinical setting and when averaged over the 24 h. In contrast, there was a direct relationship between the IMT value and the standard deviation of 24 h mean blood glucose (r=0.4, Spearman correlation, P<0.05).

Conclusions
In type 1 diabetes 24 h variability values of blood glucose seem to be a more important determinant of large artery structural alteration than fast and mean blood glycaemia.

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Relationship between nerve conduction velocity with uncoupling protein 2 promoter polymorphism -866G/A and A allele frequency in type 2 diabetic patients with diabetic peripheral neuropathy

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Progression of the diabetic peripheral neuropathy (DPN) demonstrated differences in the individual considered the contribution of genetic predisposition. We aim to study the relationship between the −866G/A polymorphism in the promoter region of the uncoupling protein 2 (UCP2) which enhances transcriptional activity and A allele frequency with nerve conduction velocities (NCV) and clinical factors. We compared 370 type 2 diabetic patients with healthy control. Subjective neuropatic symptoms, neurologic examination and electrophysiologic measurements were evaluated. Diabetic patients were divided into two groups Group 1: (G/G) and Group 2: (G/A + A/A) according to genotype. Clinical features and NCV were compared and also independent risk factors of the DPN were defined. The comparison of two independent group was performed with Mann–Whitney *U*-test or Fisher's Exact test. Relationship between NCV with UCP2 genotype and cinical factors assessed through multiple regression analysis (MRA) and ANOVA. Pearson correlation was performed for correlation analysis. DPN was present in 46.2% of the cases. DPN was significantly correlated with HbA1c, duration of DM, age at DM onset, retinopathy and nephropathy. The proportions of the individuals carrying genotype (-866G/A and A/A) and A allele frequency were significantly higher in the diabetic patients with DPN than that in the controls respectively ($P<0.009$, $P<0.004$). Group 2 had an early age at DM onset ($P<0.0001$) and high proportion of retinopathy ($P=0.03$). NCV was significantly lower in Group 2 than that in Group 1. MRA showed that UCP2 genotype and HbA1c were related with the impairment of NCV independently of other clinical factors. HbA1C, and retinopathy. In conclusion, higher A allele frequency with DPN and early age at DM onset in the Turkish diabetic population might partly indicate the genetic predisposition. Higher UCP2 activity related to A allele which was independent risk factor in the impairment of NCV may contribute to reduce NCV via decreased ATP production in mitochondria. On the other hand, increased UCP2 in the DPN may be a marker of an inadequate antioxidant effect of the UCP2 in the chronic oxidative stress. Investigation of the genetic tendency for DPN may guide to define the risk factors and also in the new insights for the treatment.

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No genetic impact of the SOCS3-gene on T2DM

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The diabetic epidemic is increasing worldwide. The suppressor of cytokine signaling 3 (SOCS3) provides a link between cytokine action and their negative consequences on insulin signaling. This potential influence on the development of T2DM makes the gene a potential candidate gene for type 2 diabetes.
We investigated the one haplotype tagging single nucleotide polymorphism (htSNP) rs4969168 to examine the impact on T2DM in two independent study populations; one prospective case-cohort study and one cross-sectional cohort. In the prospective cohort a total of 2,242 individuals (669 had developed T2DM during the follow-up period) were analyzed. In the dominant model there was no effect of the polymorphism on diabetes risk (hazard ratio (95% CI): 0.86 (0.66–1.13); $P=0.3$). In the second, cross-sectional MeSyBePo-cohort 1,897 subjects (325 had T2DM) were investigated. Accordingly to the prospective results, no association with T2DM was found (odds ratio (95% CI): 0.78 (0.54–1.12); $P=0.8$). We also calculated the power in our cohorts, to estimate the detectable effect. This resulted for the EPIC-cohort in a relative risk under 0.72 or above 1.35 and for the MeSyBePo-cohort under 0.66 and above 1.44.
In conclusion there is no strong effect of the one htSNP in the SOCS3 gene on the development of T2DM. However, the impact of genetic variants on complex diseases is usually quite small. Thus a small effect might still exist, which has not been detected by our cohorts, although a total of more than 4000 individuals was investigated.

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Atherosclerosis in premenopausal diabetic women with or without polycystic ovary syndrome

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Background and aims
It is well recognized that insulin resistance and type 2 diabetes (T2DM) are associated with an increased risk for cardiovascular disease especially in women, while the role of sex steroids is still controversial. Women with polycystic ovary syndrome (PCOS) represent a unique biological model for the study of the impact of all these factors on cardiovascular system. We aimed to evaluate the degree of low-grade inflammation and atherosclerosis in premenopausal diabetic women with or without PCOS by measurement of C-reactive protein (CRP) and common carotid intimal-medial thickness (IMT).
Subjects and methods
We studied 40 women with T2DM (mean age 43.2 years) treated with metformin and/or sulfonylourias and 30 healthy, normal cycling, age-matched women. According to gynaecological history (menstrual cycles ≤ 8 per year) and the presence of hyperandrogenism (clinical or biochemical), diabetic women were divided in two groups: group 1 diabetic women with normal cycles and group 2 diabetic women with PCOS. We measured BMI, waist circumference (WC) and systolic blood pressure (SBP). Blood samples were collected after an overnight fast, during the 2–6 day of the cycle and hormones, lipids, glucose and CRP levels were determined. Carotid IMT was assessed by B-mode ultrasound imaging.
Results
No difference was found between diabetic and control women in age, smoking habits and the presence of hypertension. Diabetic women had significantly higher BMI (34.5 ± 6.39 vs 27.9 ± 6.1, $P<0.001$), WC (105.3 ± 14 vs 86.9 ± 11.2 cm, $P<0.001$), hirsutism score (1.78 ± 0.9 vs 1.15 ± 0.3, $P<0.01$) and a positive family history for T2DM (82.5% vs 34.6%, $P<0.002$). Additionally, they presented higher total testosterone (0.62 ± 0.21 vs 0.45 ± 0.11 ng/ml, $P<0.001$), insulin (17.8 ± 11.9 vs 9.8 ± 3.6 mIU/ml, $P<0.002$), triglycerides (131 ± 23 vs 95 ± 67 mg%, $P=0.02$) and LDL-cholesterol (132 ± 31 vs 114 ± 23 mg%, $P=0.025$) levels and lower SHBG (34.3 ± 16.2 vs 53.9 ± 18.7 nmol/l, $P<0.001$) and HDL-cholesterol (47.1 ± 12.8 vs 57 ± 10.9 mg%, $P<0.001$) levels compared to control women. Between diabetic women, patients in group 2 reported higher percentage of Pregnancy Diabetes (37.5% vs 8.3%, $P<0.05$) were younger at disease diagnosis (32.8 ± 5.7 vs 39.7 ± 6.1 years, $P<0.001$) and they had higher WC (115 ± 12.8 vs 99 ± 11.1 cm, $P<0.01$). Furthermore, they presented higher total testosterone (0.75 ± 0.22 vs 0.54 ± 0.16 ng/ml, $P=0.01$) and insulin (24 ± 15.8 vs 14.4 ± 7.5 mIU/ml, $P<0.05$), and lower SHBG (26.1 ± 9.5 vs 39.8 ± 17.6 nmol/l, $P<0.001$) and HDL-cholesterol (41.4 ± 11.2 vs 50.8 ± 12.6 mg/%, $P<0.001$) levels. Duration of T2DM and glycaemic control was the same in both groups but women in group 2 were younger (39.9 ± 6.3 vs 44.5 ± 3.6 years, $P<0.02$). Diabetic women had higher CRP levels (1.4 ± 0.4 vs 0.8 ± 0.2 mg/dl, $P<0.05$) and carotid IMT (0.065 ± 0.007 vs 0.056 ± 0.008 mm, $P<0.001$) compared to controls, while no difference was found between the two groups of diabetic women.
Conclusions
Premenopausal diabetic women have low-inflammation and accelerated atherosclerosis. High androgen levels do not seem to aggravate this process as in the case of diabetic women with PCOS.

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Effects of testosterone and sildenafil on cytokines and angiogenic factors: a randomized controlled trial

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Ageing in men is accompanied by a decline in serum testosterone (T) levels and increased risk of cardiovascular disease, osteopenia, visceral obesity and impaired metabolism. These conditions have complex aetiologies that may share a common pro-inflammatory state. We investigated the impact of T therapy on cytokines and angiogenic factors involved in atherosclerosis and bone loss, in hypogonadal men who underwent androgen replacement. We carried out a 6 months, randomized, double-blind, controlled, crossover trial of T gel (50 mg/d) + Sildenafil(S) (50 mg) vs Placebo(P) + (S) in a group of 25 men (age, 52 ± 6 years) with hypogonadism and erectile dysfunction. All patients had penile CDU, IIEF, CES-D, AMS and EDITS questionnaires, DEXA for BMD, routine biochemistry and measurement of serum cytokines (IL-1, IL-6, IL-10, IL-12, TNF) and angiogenic factors (FGF, PDGF, VEGF, HGF) evaluated at baseline and at the end of each treatment period. In the T + S arm serum T levels rose from 2.6 ± 2.3 to 3.8 ± 2.2 ng/dl ($P < 0.05$) with improvement in penile Peak Systolic Velocities (from 26 ± 7.3 to 34 ± 8.9 cm/s, $P < 0.01$). T + S also induced a significant reduction in serum pro-inflammatory IL-1β (−27 ± 8%; $P < 0.05$) and TNF-α (−23 ± 6%; $P < 0.05$) and an increase in anti-inflammatory IL-10 (45 ± 9%; $P < 0.05$). Sildenafil alone induced a reduction of FGF and VEGF, compared with the association of T + Sildenafil (respectively, −18 ± 8% and −22 ± 9%, $P < 0.05$), while the reduction in PDGF was inconsistent (−13 ± 8%, $P = 0.05$). These data suggest that: 1) T in men plays an immune-modulatory effect shifting circulating cytokine toward a favourable anti-inflammatory state; 2) PDE5 inhibitors (PDE5i) reduces markers of vascular damage; 3) the association of T and PDE5i in hypogonadal subject could potentiate the positive endothelial and vascular effects of PDE5i and counteract the pro-angiogenic effects of T. These data disclose future indications for the combined treatment of androgen and PDE5i in the rehabilitation of patients with hypogonadism and cardio-vascular disease.

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Smoking shortens insulin-free interval in type 2 diabetics

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Smoking is a modifyable risk factor in the incidence of impaired glucose tolerance and type 2 diabetes, but the data concerning the effect of smoking on the course of type 2 diabetes is limited. In this study, we aimed to investigate a possible relationship between smoking and the need of insulin therapy in patients with type 2 diabetes. 470 patients with type 2 diabetes were assessed for smoking habitus, marital and educational status, duration of diabetes, duration of insulin-naive interval, alcohol consumption, adherence to treatment, body-mass index (BMI) and HbA1c. The nonsmokers both never and former were significantly older than the smokers' group. Duration of diabetes was significantly longer in nonsmokers than smokers. Insulin treatment was more often in smokers than nonsmokers. The smokers had significantly higher HbA1c levels than both never- and former-smokers. Never smokers and former smokers had similar HbA1c levels. Duration of diabetes was similar in both groups and insulin-naive interval was significantly shorter in smokers and former smokers than never-smokers. Adherence to treatment was better in smokers than nonsmokers. BMI was significantly higher in nonsmokers than smokers. Alcohol consumption was reported in smokers, but not in nonsmokers. In conclusion, the need for insulin treatment was more frequent and earlier in smokers, which could be explained by hyperglycemia induced by smoking and increased toxicity in the microenvironment of pancreatic islets.

P205

Glycemic variability analyze with different glucometers

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Introduction
Currently self-glycemic control by the patient is essential to achieve a right metabolic control of diabetes. Nowadays, there are different systems that have proved useful for this aim.
Objectives
Valuing any possible differences between systems and the relation with glycemia identified in our laboratory.
Material and methods
We took blood samples by vein-puncture to 41 patients, and simultaneously we measured capillary glycemia using 6 different glucometer (A, B, C, D, E and F). We determined the glycemia by the method GOD-PAP (glucose oxidase).
Results
Correlation between glucometer and laboratory test (L):
L-A: 0.980; L-B: 0.979; L-C: 0.986; L-D: 0.973; L-E: 0.977; L-F: 0.89
Capillary glycemia average:
A: 131.9 ± 56; B: 139 ± 61; C: 124.4 ± 64; D: 139 ± 64; E: 127.8 ± 69.6; F: 141.9 ± 66.4.
Blood glycemia average differences between laboratory test and differents glucometers:
L-A: −50.7 ± 43; L-B: −28.37 ± 27.6; L-C: −19.1 ± 35.2; L-D: −62.95 ± 41.57; L-E: −16 ± 20; L-F: −78.56 ± 54.8. All of them had significant difference ($P < 0001$).
Capillary glycemia average differences between several glucometers:
Statistically significant differences were obtained from the following glucometers: A and B: −7.07 ± 11.8 ($P < 0.001$); A and C: 7.55 ± 13.6 ($P < 0.001$); A and D: −7.12 ± 13.23 ($P < 0.01$); B and C: 14.62 ± 11.2 ($P < 0.001$); B and E: 11.2 ± 17 ($P < 0.001$); B and F: 8.74 ± 14.5 ($P < 0.01$); C and D: −14.67 ± 12.37 ($P < 0.001$); C and F: −5.06 ± 13.27 ($P < 0.05$); D and E: 11.25 ± 17 ($P < 0.001$); D and F: 8.74 ± 14.54 ($P < 0.01$).
Conclusions
1. All glucometer had an excellent correlation with blood glycemia obtained in our laboratory (> 0.9) but our blood glucose levels were significantly lower.
2. We found significant differences in capillary glycemia values obtained with different glucometers which is important for an estrict metabolic control.
3. Glucometer E gives closer values to our laboratory ones and introduces lower variability.

P206

Evaluation of continuous subcutaneous insulin infusion in type 1 diabetes

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Objective
To evaluate continuous subcutaneous insulin infusion (CSII) in type 1 diabetes using the analysis of epidemiological variables, treatment indications, metabolic results, acute and chronic complications and causes for therapy suspension.
Methods
A retrospective descriptive study of patients with type 1 diabetes treated with CSII therapy in our institution.
Results
A total of 49 type 1 diabetic patients who had been treated with CSII were evaluated (age: 31.9 ± 8.3 years; female: 67.3%, time of evolution of diabetes: 16.5 ± 8.1 years). Before starting CSII, retinopathy was present in 46.9%, microalbuminuria in 8.2%, macroalbuminuria in 4.1%, polineuropathy in 2.0% and vasculopathy in 2.0% of the subjects. The indications for CSII treatment were: brittle diabetes (38.7%), poor metabolic control (32.6%), pregnancy (16.3%), pregnancy planning (8.2%) and hypoglycemias (4.1%). Patients received CSII therapy during 22.2 ± 12.1 months. Hemoglobin A1c before starting CSII therapy was 8.3 ± 1.2% and improved significantly during treatment: 7.1 ± 0.9% after 3 months, 7.3 ± 1.2% after 9 months, 7.4 ± 0.9% after 12 months, 7.6 ± 1.4% after 18 months and 7.5 ± 1.4% after 24 months of CSII therapy ($P < 0.001$). CSII also reduced the frequency and severity of hypoglycemias. During CSII therapy 3 patients had diabetic ketoacidosis (6.1%), 4 developed retinopathy (8.2%), 3 microalbuminuria (6.1%) and in 5 patients the previous retinopathy progressed (10.2%). Seven patients discontinued CSII: 3 for patient wish, 3 due to the end of pregnancy and one because treatment failure.
Conclusions
The most frequent indications for CSII therapy in patients with type 1 diabetes in our institution are brittle diabetes and poor metabolic control, and the most frequent causes for therapy suspension are the patient wish and the end of pregnancy. CSII is a safe and effective treatment to optimize metabolic control in type 1 diabetic patients.

P207

Elevated plasma fatty acid levels are associated with myocardial triglyceride accumulation and diastolic dysfunction in type 2 diabetes mellitus which can be reversed by acipimox

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Background
Although it has been conceived that type 2 diabetes mellitus (DM2) is associated with increased myocardial triglyceride (TG) accumulation, it is unknown whether this can be influenced by modifying plasma non-esterified fatty acids (NEFA) levels. We therefore determined in uncomplicated DM2 whether modifications in plasma NEFA influence myocardial TG content and myocardial function.
Research design and methods
Myocardial TG content and left ventricular (LV) function were determined using proton magnetic resonance spectroscopy and MRI respectively at 1.5 T (Gyroscan ACS/NT15, Philips) in 11 subjects with uncomplicated DM2 (mean age ± S.D.: 59.8 ± 5.4 years, BMI 26.2 ± 0.9 kg/m^2, HbA1c: 6.0 ± 0.2%) before and after 3 days of a very low calorie diet (VLCD, 473 kcal/day, to increase plasma NEFA levels) and after 3 days of a VLCD complemented with the administration of acipimox (4 × 250 mg during the last day of caloric restriction, to decrease NEFA levels). The percentage (%) of myocardial TG was calculated as TG/water × 100. Myocardial function was calculated as ejection fraction (EF) for systolic function, and the ratio between early and atrial filling phase (E/A ratio) for diastolic function.
Results
A VLCD increased plasma levels of NEFA, from (mean ± S.E.M.) 0.50 ± 0.08 mmol/l (baseline) to 0.91 ± 0.15 ($P = 0.023$), associated with myocardial TG accumulation (from 0.66 ± 0.09% (baseline) to 0.98 ± 0.16% ($P = 0.028$), and a decrease in E/A ratio (from 1.00 ± 0.05 (baseline) to 0.90 ± 0.06 ($P = 0.002$). After the VLCD with acipimox, plasma NEFA levels decreased (to 0.24 ± 0.05 mmol/l, $P = 0.002$) and myocardial TG content and diastolic function returned to baseline values.
Conclusion
A short-term VLCD induces myocardial TG accumulation in patients with uncomplicated DM2, and is associated with changes in diastolic function. Administration of acipimox during the VLCD reduced myocardial TG accumulation together with plasma NEFA level in conjunction with diastolic heart function. These data stress the relation between plasma NEFA levels and myocardial TG accumulation in DM2.

P208

May testosterone therapy be the key to metabolic syndrome (MS) treatment in men?

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Background
Impaired glycaemic control, resulting in diabetes mellitus type 2 (DMT2), is one of the MS components. In 1998 the UKPDS study showed that over 10 years of observation there were no significant changes in haemoglobin A1c (HbA1c) in intensive insulin therapy group compared with conventional therapy group. Therefore, we still need new options to treat DMT2. Testosterone is well-known for it's lipolytic activity; obesity leads to insulin resistance and DMT2.
Objective
To study the glycaemic status in men with androgen deficiency (AD), MS and DMT2, treated with testosterone/placebo.
Materials and methods
Twenty-eight men with MS (IDF criteria), DMT2 (nine patients were on insulin therapy) and AD (total testosterone (TT) < 11 nmol/l) received 3 injections of testosterone undecanoate (Nebido, Schering) or placebo. Patients were divided into two groups according to TT levels 12 weeks after the third injection of testosterone/placebo: in group 1 ($n = 17$) TT was normalized up to 15.8 (13.0–21.0) nmol/l and in group 2 ($n = 11$), TT was not normalized (8 (6.5–10.3) nmol/l (normal range (NR) 11–33)). Fasting plasma glucose (FPG) was measured before and after 30 weeks. Statistical analysis was performed using Wilcoxon test.
Results
In group 1, FPG decreased from 6.7 (6.3–9.3) to 6.3 (5.3–877) ($P = 0.01$). In three patients, insulin was no longer needed. In group 2, FPG increased from 6.71 (6.4–7.0) to 7.0 (6.1–7.5), but these changes were not significant ($P = 0.53$).
Conclusion
AD correction in men with MS and DMT2 improves glycaemic parameters.

P209

Relationship of insulin sensitivity with carotid intima media thickness in patients with type 2 diabetes mellitus

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Objective
The study was to establish the association between insulin sensitivity (IS) and carotid intima media thickness (CIMT) in type 2 diabetic patients (T2D pts).
Materials and methods
40 (21 f,19 m) T2D pts aged 58.1 ± 10.2 years, BMI 30.1 ± 5.8 kg/m^2 (mean ± S.D.); 36 (19 f, 17 m) normal glucose tolerant (NGT) subjects of mean age 57 ± 10.1 years, as a control group in terms of IS, and 38 (20 f, 18 m) NGT subjects of mean age 57.6 ± 9.4 years, as a control group in terms of CIMT, participated in the study. IS was measured with a manual hyperinsulinaemic euglycaemic clamp technique and expressed as a glucose disposal rate (M; mg/kg per min). CIMT was assessed using B mode sonograph 'Ultrasonix', M'Ath Software in both right common carotid artery (CCAr) and left common carotid artery (CCAl). The study was approved by Local Ethical Committee.
Results
IMTCCAr 0.76 ± 0.15 mm and IMTCCAl 0.82 ± 0.21 mm of T2D pts were significantly higher than IMTCCAr 0.59 ± 0.12 mm and IMTCCAl 0.58 ± 0.10 mm of NGT subjects, both $P < 0.001$. IS (M) of T2D pts significantly inversely correlated with IMTCCAr $r = -0.51$ and IMTCCAl $r = -0.57$, both $P < 0.001$, independently of age, BMI, waist circumference, blood pressure, triglycerides, LDL cholesterol and HDL cholesterol. According to the median level of M 6.118 mg/kg per min of NGT subjects, T2D pts were divided into insulin sensitive (IS) and insulin resistant (IR). There was a significant difference between IMTCCAl 0.87 ± 0.20 of IR pts and IMTCCAl 0.62 ± 0.23 of IS pts, $P < 0.01$. There was a significant difference in CIMT between IR pts and NGT subjects, whereas we did not find a significant difference in CIMT between IS pts and control group.
Conclusion
Insulin resistance could be considered as an important factor for increased carotid intima media thickness in type 2 diabetic patients.

P210

Diabetic survival with HD: 10-years follow-up

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Background
Diabetic nephropathy affects 20–40% diabetics. It is the most common cause of ESRD in Europe. In Serbia, 10–18% of the patients requiring HD are diabetics and that number is constantly increasing.
Methods
We made a retrospective study of the survival of both diabetic and non-diabetic patients requiring HD treatment during two 5 years long periods.
Results
During the first period 92 patients were followed-up and during the second 93 patients. There was not any statistically significant age difference between diabetics and non-diabetics with HD therapy, and between disease duration before HD therapy among diabetics type 1 vs type 2, in none of the analysed periods. During the first period survival of diabetics on HD was shorter at a highly statistically significant level compared to non-diabetics 2.3 ± 2.6 years for DM compared to 4.7 ± 3.9 years for non-DM, but it was not statistically significant when we compared patients with type 1 and type 2 DM. During the second period survival of patients with HD therapy was highly statistically shorter for diabetics vs non-DM 1.4 ± 1.5 vs 4.1 ± 2.7 years, and for DM type 2 compared to type 1 DM 0.9 ± 0.4 vs 2.8 ± 2.3 years. Between 1991 and 1995, 19 diabetics – 21% started HD therapy, between 1996 and 2000, 14 diabetics – 15% and between 2001 and 2005, 28 diabetics – 22% started HD therapy. That increase is especially significant in 2005, 12 out of 21–57%.
Conclusion
Prognosis for ESRD patients caused by diabetic nephropathy is extremely bad. That is why all preventive measures should be taken, not only regarding the basic disease, DM, but also in terms of primary and secondary prevention of diabetic nephropathy.

P211

Markers of oxidative stress and antioxidant status in patients with gestational diabetes

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Objective
Evaluate the markers of oxidative stress and antioxidants status in patients with gestational diabetes (GD).
Material and methods
We performed a nested case-control study within a sample of a total of 126 pregnant women between the 24 and 29 weeks. Of the 126 women studied we compared 63 with GD and 63 control pregnant women. Both groups were analyzed for demographic data, perinatal and obstetrics results and the levels of the markers oxidative stress and antioxidants status (lipid peroxides, glutathione peroxidase, glutathione reduced, catalase and superoxide dismutase).
Results
Maternal age studied for the control group was 29.95 ± 5.03 years old and 31.57 ± 4.1 years in patients with GD. Results showed 23% of the control group and 26% in patients with GD presented a history of abortion or macrosomia. The pre-pregnancy BMI was 23.46 ± 3.73 kg/m^2 in the control group and 28 ± 3.6 kg/m^2 in GD ($P=0.0001$).
In reference to the perinatal and obstetrics results, the final week of pregnancy was 39.62 ± 1.2 in the control group and 39.02 ± 1.8 in GD. The rate of cesarean delivery was 10% in the control group and 33% in the patients with GD ($P=0.0001$). The rate of macrosomia was 8% in the control group and 16% in GD ($P=0.3$).
Levels of lipid peroxides ($P=0.001$), peroxidase glutation ($P=0.01$), catalase ($P=0.04$) and dismutase superoxide ($P=0.0001$) were statistically siginificant. In the multivariance analysis the levels of lipoperoxides ($P=0.03$), catalase ($P=0.006$) and superoxide dismutase ($P=0.003$) were significantly associated with the dependent variable GD.
Conclusions
Women with GD present a higher probability of developing higher risk markers of cardiovascular disease expressed as higher levels of lipid peroxides and descending levels of antioxidants such as the catalase and superoxide dismutase.

P212

Adipokine profile in gestationa diabetes mellitus

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Objective
Evaluate the proinflammatory and anti-antinflamatory adipokines in patients with gestational diabetes (GD).
Material and methods
We performed a nested case-control study within a sample of a total of 126 pregnant women between the 24 and 29 weeks. Of the 126 women studied we compared 63 with GD and 63 control pregnant women. Both groups were analyzed for demographic data, perinatal and obstetrics results and the plasma levels of the tumor necrosis factor (TNF-α), Interleukin (IL-6), adiponectin and leptin.
Results
Maternal age studied for the control group was 29.95 ± 5.03 years old and 31.57 ± 4.1 years in patients with GD. Results showed 23% of the control group and 26% in patients with GD presented a history of abortion or macrosomia. The pre-pregnancy BMI was 23.46 ± 3.73 kg/m^2 in the control group and 28 ± 3.6 kg/m^2 in GD ($P=0.0001$).
In reference to the perinatal and obstetrics results, the final week of pregnancy was 39.62 ± 1.2 in the control group and 39.02 ± 1.8 in GD. The rate of cesarean delivery was 10% in the control group and 33% in the patients with GD ($P=0.0001$). The rate of macrosomia was 8% in the control group and 16% in GD ($P=0.3$).
Levels of TNF-α ($P=0.002$), leptin ($P=0.001$) and adiponectin ($P=0.04$) were statistically significant. In the multivariance analysis the levels of adiponectin ($P=0.026$) and the pre-pregnancy BMI (0.01) were significantly associated with the dependent variable GD.
Conclusions
Women with GD present a higher probability of developing higher risk markers of cardiovascular disease expressed as a higher pre-pregnancy BMI and descending levels of adiponectin.

P213

Patients with type 2 diabetes mellitus have intima media thickness of carotid artery wall equal to that of a decade older non-diabetic patients

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Background
High resolution real-time B-mode ultrasound diagnostic and off-line computerized measurement of vasculature can precisely assess the intima-media thickness (IMT) of the vascular wall and the presence of atherosclerotic plaque.
Aim of the study
To determine whether there is a difference in IMT of common carotid artery wall (CCA) between patients with diabetes mellitus type 2 (DM2) and non-diabetics (nonDM).
Materials and methods
Three hundred and sixty patients are examined – mean age 55.47 ± 11.97 year, male – 120, female 240, DM2 – 195, non-diabetic – 175. The difference in age between diabetics and non-diabetics with equal IMT measure is calculated. IMT was assessed by sonograph 'Ultrasonix', linear transducer 7.5–12 MHz and M'Ath Software.
Results

	Male DM2	Male non-DM	Female DM2	Female non-DM
IMTcca-left (mm)	0.747	0.675	0.727	0.605
IMTcca-right (mm)	0.785	0.675	0.758	0.617

Linear regression analysis (Stepwise method).
IMTcca-left $= 0.229 + 0.008 \times$ Age $+ 0.105 \times$ DM – male – 13.1 years.
IMTcca-left $= 0.244 + 0.007 \times$ Age $+ 0.100 \times$ DM – female – 14.3 years.
Age (years), DM = 1 for DM2, DM = 0 for nondiabetic.
Conclusions
Male with DM2 have IMTcca-left equal to that of non-diabetic male that are 13.1 year older than them. Women with DM2 have ITMcca-left equal to that of non-diabetic female that are 14.3 year older than them. IMT of the left CCA is significantly thicker than IMTcca-right. The presence of diabetes mellitus precipitates early thickening of the vascular wall.

P214

Kidney transplantation and diabetes mellitus: continuous monitoring of glucose

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Introduction
The benefits of intensive management of type 1 and type 2 diabetes have been well established in several studies, as DCCT and UKPDS, and include reduced of long term complications. Current guidelines target of A1c $<7\%$ is also to achieve in diabetic patient with kidney transplant. Methods to improve patient's ability to achieve this goal are being explored. Continuous monitoring of glucose in interstitial fluid allows the identification of glycemic excursions and general patterns of glucose levels in a manner not available with self-monitoring blood glucose. Knowledge of these excursions and patterns may be used to make appropriate changes in treatment of diabetes.
Patients and methods
Continuous monitoring of glucose in interstitial fluid with MiniMed-CGMS was performed in two patients with type 1 diabetes and a kidney transplant. Both patients had A1c $>7\%$ and were under intensive therapy.
Results
Reported CGMS download were analyzed. We identified 'down phenomen', undetectable hypoglycaemia events, incorrect adjustments insulin therapy… Therapy adjustments based on this information were done. No mechanical

device or local complaints were reported. After six month, glycemic control improved (A1c $<$ 7%).
Conclusions
Continuous monitoring of glucose is an important tool in intensive management of diabetes. This technology should be performed in all diabetic patients when goals are not achieved. It helps health team to make therapy adjustments and can improve patients' glycemic control. This is the way to stopped progression of macro and microvascular complications that are present in diabetic patients as those with kidney transplant.

P215

Metabolic changes induced by glucose infusion in persons with Glu23Lys polymorphism

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The development of diabetes mellitus type 2 (T2DM) is affected by genetic and lifestyle factors. Polymorphisms within the KIR6.2 gene seem to be associated with a higher risk for T2DM. The protein encoded by this gene represents a potassium channel, which plays an essential role in insulin and glucagon secretion by the pancreas.
A hyperglycaemic clamp without insulin was performed over 2 h in 10 healthy persons with the Kir6.2 E23K wild type Glu/Glu, 10 age-, BMI- and sex-matched persons being heterogenous at Kir6.2 E23K (Glu/Lys) and 10 persons with the Lys/Lys genotype. Effects on insulin and glucagon secretion were analysed.
Insulin values were not different, although the maximum peak of the insulin answer was delayed for the Lys/Lys group (Glu/Glu-peak: 2.5 min, Lys/Lys-peak: 5 min). Glucagon was at baseline significantly different between Glu/Glu (39.1 ± 2.9 pg/ml) and Lys/Lys (30.3 ± 1.6) individuals ($P = 0.015$). In addition, Glucagon decreased significantly in the Lys/Lys group after 2 h ($85.9 \pm 2.3\%$; $P < 0.001$) and the AUC values for the glucagon time course showed significant lower results (4218 ± 332 vs 3321 ± 194; $P = 0.035$) compared to the Glu/Glu group.
In conclusion we demonstrated in this study for the first time lower glucagon levels in healthy persons with the Glu23Lys variant in the KIR6.2 gene. In addition a stronger reduction in glucagon suppression after i.v. glucose was found. These results in healthy individuals indicate that specifically effects on glucagon secretion might contribute to the development of type 2 diabetes.

P216

Effects of testosterone administration in male patients with heart failure with and without hypogonadism

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Background
Testosterone administration (TA) can improve exercise capacity and glucose metabolism of males patients (pts) with chronic heart failure (CHF). It is not known whether testosterone supplementation benefits only CHF subjects with hypogonadism.
Aim
To compare effects of TA in male pts with CHF with and without testosterone deficiency.
Methods
Fifty-six males patients (median age 64.2 ± 9.6), ejection fraction (EF) 36 ± 9.4, NYHA class II/III (31/25), were enrolled. Of these 38 were randomized to receive TA, intramuscular injection of testosterone undecanoate, every 6 weeks (Nebido, Bayer Shering Germany) and 18 to receive placebo both on top of maximal medical therapy. Treated patients were than divided, according to median value of testosterone, into two groups with Low (LT = 14 pt) or high (HT = 24 pt) testosterone levels. At baseline and after 12 weeks all patients underwent glycometabolic assessment through HOMA index, cardiopulmonary test, 6-minute walking test (6MWT) and quadriceps maximal isometric and isokinetic strength.
Results
At baseline LT group had a significant lower peak oxygen uptake (VO_2) (-2 ± 0.6; P 0.01) and had shorter exercise time (-2.6 ± 1.3; P 0.04) than HT pts and placebo group. After three months LT group had a significant greater improvement from baseline of peak VO_2 (18% vs 9%; P 0.02), VE/VCO_2 (-14% vs -4%; P 0.01) HOMA index (-16% vs -4%; P 0.03) and maximal isometric strength (19% vs 2%; P 0.03) than HT group. Distance walked at 6MWT and isokinetic strength improved in a similar rate in both LT and HT groups ($P > 0.05$). There was not significant change in any endpoint in the placebo group.
Conclusion
Effects of TA in male with CHF on both exercise capacity and glucose metabolism seem to be greater in those pts with testosterone deficiency.

P217

GC-tofMS metabolite profiling in the pancreatic beta cell line INS-1 under chronic glucose stress conditions

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Background
Long term exposure of INS-1 beta cells to elevated glucose concentrations has been observed to impair insulin expression, a phenomenon termed as glucose toxicity. Despite its potential relevance in the progression of diabetes, the underlying mechanisms is not yet fully understood.
Aim
Target analysis of single, or a sub-group of metabolites has until now been the method of choice in mammalian cells to investigate the pathological state in terms of small molecule composition. We therefore aimed to show that metabolite profiling is useful to detect multitude changes in metabolism in INS-1 cells after chronic high glucose exposure. We further suggest that mitogen-activated protein kinases (MAPK) participate in the regulation of insulin gene expression.
Methods
INS-1 cells were incubated with medium containing 3 mM and 16 mM glucose $\pm$ MAPK inhibitor for 48 h. Insulin mRNA was measured using quantitative real time RT-PCR. Intracellular metabolites were extracted with a chloroform:methanol:water mixture and analysed by GC-tofMS.
Results
Insulin gene expression is significantly reduced after exposure of INS-1 cells to chronic high glucose levels. The application of INS-1 extracts to GC-tofMS allowed the relative quantification of several hundred biological peaks. The use of principal component analysis demonstrates a clear differentiation of cells treated with low or high glucose. Further in-depth analysis revealed details of glucose-derived changes in single metabolites. The inhibition of MAPK results in a restoration of insulin gene expression at high glucose levels that may be explained due to improvement of specific metabolite levels.
Conclusion
Metabolite profiling allowed the identification of core metabolites which were present at significantly different levels in low and high glucose treated INS-1 cells and which may participate in the regulation of insulin gene expression.

P218

Diabetes management and metabolic control are below expectations in specialist diabetes practice in Portugal

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In Portugal, no relevant epidemiologic data exists relative to the management and level of control of diabetic patients.
TEDDI, a non-interventional cross-sectional study, allowed to characterize the usual management of type 1 (T1D) and type 2 (T2D) diabetes using a standardized questionnaire in 1775 patients (F: 47.5%; T2D: 80.8%) aged 18 years or older, visiting their diabetes specialist ($n = 180$).

Data was obtained for metabolic control and risk factor prevalence. Main results are as follows (CI 95%):

n	HTN, %	High LDL, %	Low HDL, %	High TGs, %	non-FBG, mg/dl	HbA1c, %
T1D, 336	29.9 (25.2–35.0)	30.8 (26.0–36.0)	36.6 (31.5–42.0)	16.5 (12.9–20.9)	176.4 (±85.9)	8.0 (±1.7)
T2D, 1395	70.1 (67.7–72.4)	51.2 (48.5–53.9)	40.6 (38.0–43.3)	35.0 (32.5–37.5)	183.7 (±73.0)	7.6 (±1.5)

Severe hypoglycaemic crises (requiring emergency care) were reported by 3.4% of patients (T1D: 7.5%; T2D: 2.3%) in the preceding month. In these, an average of 2.4 crises per patient was determined.
In T2D, level of insulinization was 47.0%, and 31% were treated simultaneously with oral hypoglycaemic, antihypertensive, antidyslipidemic and anti-platelet drugs. In T1D, 16.1% of patients used LA insulin, while metformin was used as an adjuvant therapy in 15.2%.
Although old barriers in diabetes management (insulin for T2D and LA insulin and metformin for T1D) are overcome, metabolic control is still below expectations.

P219

Glucose metabolism alterations in non-diabetic patients undergoing cardiac rehabilitation programs

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Background
Effects of cardiac rehabilitation (CR) on glucose metabolism of coronary artery disease (CAD) patients (pts) without diabetes has not been assessed.
Aim
Evaluate effects of an intensive CR program on glucose metabolism of no-diabetic CAD pts with.
Methods
Sixty non-diabetic pts (M/F 53/7), age 71.2±9.2, were submitted to a 3 week CR program. Oral glucose tolerance test (OGTT), HOMA index, six minute walking test (6mwt) were performed at baseline (T0), three weeks (T1) and after three months (T2). At discharge a dietetic and exercise program was recommended.
Results
At T0 28.3% had normal glucose tolerance, 41.6% IGT, and 30.1% were diabetic (DM). At T1 there was a significant reduction of BMI (29.8±3.4 vs 29.0±3.8 *P* 0.001), waist circumference (81.0±34.1 vs 79.1±33.4 cm *P* 0.006), HOMA-R (2.6±1.4 vs 1.8±0.8, *P* 0.002). 64.7% of previous diagnosed IGT pts had normal glucose tolerance (*P* 0.04), and 77% of previous DM resulted IGT. At T2 BMI, fasting glicemia, HOMA-R increased to similar values than baseline.
Overall at T1 distance walked at 6MWT improved significantly (287±107.9 vs 482±117.8, *P* 0.001). IGT pts had a worst performance than pts with normal glucose tolerance at baseline and discharge 6MWT (291.1±83.4 vs 344.4±87.1; $P<0.04$; and 444.9±102.3 vs 510.7±84.7, $P<0.02$ respectively). No difference was found between IGT and DM pts at baseline (291.1±83.4 vs 264.7±86.3 m; $P>0.05$) and discharge 6MWT (444.9±102.3 vs 457.8±100 m, $P>0.05$).
Conclusion
OGTT is usefull to identify gluco-metabolic state in CAD pts. IGT pts have a worse functional capacity than normo-glicemic. CR program improves glucose metabolism and insulin resistance in cardiac pts with impaired OGTT. However results are lost when CR is stopped.

P220

GDM in women younger and older than 28 years: are there any differences in phenotype and biochemical markers?

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Goal
The aim of this study was to find different characteristics between GDM women aged under (GDM A $n=23$) and above 28 years (GDM B $n=107$), followed in our department in 2005.
Material and methods
We performed ANOVA comparisons and computed Pearson correlations/linear regressions between womens' age and BMI, O' Sullivan test, OGTT 0 h, 1 h, 2 h and 3 h, new born weight and the need of insulin.
Results
BMI was similar in both groups (mean value $A=24.6$ kg/m^2, $B=24.9$ kg/m^2, *P* ns). O'Sullivan test measures were significantly higher in group *B* as compared to group *A* (mean values $B=166$ mg/dl, $A=151$ mg/dl, $P=0.04$). There was a positive correlation ($r=0.272$) between these measures and age ($P=0.028$). We found no significant differences across OGTT measures between these groups. Birth weight was significantly lower in group *B* compared to group *A* (mean values $B=3.190$ kg, $A=3.480$ kg, $P=0.03$). Insulin therapy was given more often in group *B* as compared to group *A* (35.5% and 8.6% respectively, $P=0.011$, χ^2 test).
Conclusions
Age was not correlated with BMI and OGTT for any of the groups.
The older group had significantly higher levels of O'Sullivan test. Maternal age influenced the need for insulin. In the older group birth weights were lower, probably due to the better glycaemic control, given the more aggressive insulin therapy.

P221

SelS/Tanis controls insulin sensitivity and is regulated by acute phase and diet

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Background
Selenoproteins contain the 21st proteinogenic amino acid selenocysteine (Sec) and exert important biological functions. Selenoprotein S (SelS) has only recently been identified and appears to be implicated in type 2 diabetes and inflammation. *In vitro* studies have shown that SelS expression is controlled by circulating cytokine and glucose levels. Mechanistically, SelS participates in the retro-translocation during quality control in the ER and the ER-associated degradation pathway.
Hypothesis
SelS is a selenoprotein and as such its translation depends on the selenium (Se) supply. mRNA levels may fail to reliably reflect SelS protein levels, especially if the organism is stressed by an acute phase response (APR). Therefore, Se-deficiency and/or APR may impair the regular SelS expression.
Material and methods
We have injected male and female mice on different Se status with lipopolysaccharide (LPS) to induce an APR. SelS levels were determined by qPCR and Western blot analysis in liver, fat and muscle.
Results
SelS biosynthesis turned out to be intensively regulated on the posttranscriptional level by both the APR and the Se status of the organism. In liver, fat and muscle tissue, we observed a tissue-specific increase of SelS protein concentrations in well-supplied animals (up to 20-fold induction of SelS protein) but only marginal effects on SelS mRNA. This Se-dependent expression was characteristically altered during the APR.
Conclusion
Since SelS is involved in glucose metabolism, diabetes and oxidative stress defence, sufficient dietary Se is needed for the regular biosynthesis of SelS, and thereby may impact onto the individual insulin sensitivity and APR.
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P222

Atherosclerotic factors, low antioxidant defense and cardiovascular diseases in retinal occlusions and anterior ischemic optic neuropathy

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Aim
The aim of the study was to examine the existence of cardiovascular diseases (CVD), atherosclerotic factors and antioxidant defense in patients with central retinal artery and central retinal vein occlusions (CRAO, CRVO) and anterior ischemic optic neuropathy (AION).

Materials and Methods
The study included 38 obese individuals with CRAO, CRVO and AION. Glycoregulation disorders were determined by OGTT. Lipid status was determined by spectrophotometry. Serum CRP was measured by immunometric assay. Microalbuminuria was determined by nephelometry. Activities of markers of antioxidant defense, superoxide dismutase (SOD) and glutation peroxidase (GSH-Px) were determined in erythrocytes.
Results
Disorder percentages in observed patients were as follows: CRAO 46.4%, CRVO 35.7%, AION 17.9%. CRAO, CRVO and AION patients had the following respective values of the following disorders: hypertension 63.3%, 60.0%; 80.0%; obesity 69.2%, 70.0%, 60.0%; hyperlipoproteinemia 84.6%, 100.0%, 80.0%; metabolic syndrome (MS) 69.2%, 70.0%, 80.0%; angina pectoris 30.8%, 10.0%, 40.0%; miocardial infarction 0%, 10%, 40%; impaired fasting glucose (IFG) 30.8%, 10.0%, 20.0%; diabetes mellitus type 2 (T2DM) 15.4%, 40.0%, 60.0%. LDL/HDL ratio was 3.3 ± 1.4, 3.3 ± 0.7 and 3.0 ± 0.7. Lp(a) was 0.37 ± 0.77, 0.43 ± 0.38 and 0.13 ± 0.1. Microalbuminuria was 21.7 ± 14.7, 112.9 ± 166.9 and 13.0 ± 4.8. CRP was highest ($P<0.01$) in CRAO patients (3.7 ± 1.4; 1.9 ± 1.0; 0.9 ± 0.7 mg/l). Decreased antioxidant defense was present in all disorders.
Conclusions
Visceral obesity, hypertension, hyperlipoproteinemia are associated with accelerated atherosclerosis and CVD, which was most frequent in CRAO (highest CRP as inflammatory factor, highest atherogenic risk factor LDL/HDL, high Lp(a)) and AION patients (most frequent T2DM with poor glycoregulation and MS with all its characteristics). Low antioxidant defense is associated with atherosclerosis and various vascular complications.

P223

Glucagon-like peptide 1 and glucose-dependent insulinotropic polypeptide in polycystic ovary syndrome

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Insulin hypersecretion during oral or intravenous glucose tolerance test (OGTT, ivGTT) was described previously in women with polycystic ovary syndrome (PCOS). Little attention was given to the regulation of insulin secretion in these subjects. We aimed to study the secretion of incretins (glucose-dependent insulinotropic polypeptide, GIP, glucagon-like peptide 1, GLP-1), during OGTT in normoglucose-tolerant PCOS women.
After signing written informed consent approved by the local Ethical Committee, women with PCOS ($n=21$,age 25.8 ± 4.1 years, BMI 21.6 ± 1.7 kg/m^2) and control healthy women (CT $n=13$, 28.5 ± 7.2 years, 20.3 ± 2.5 kg/m^2) underwent OGTT with sampling for blood glucose, insulin, C-peptide, total GIP and active GLP-1. Insulin sensitivity was assessed in fasting (QUICKI) and in dynamic conditions (OGIS; ml/min per m^2). Two-sample *t*-tests or repeated measures ANOVA followed by LSD were done. Values are given as mean$\pm$S.D.
Basal glucose, insulin and C-peptide did not differ between PCOS and CT. Similarly, stimulated glucose and insulin did not differ significantly between PCOS and CT as well as QUICKI and OGIS. During OGTT, PCOS had higher levels of C-peptide than CT irrespective time (ANOVA PCOS vs CT $P<0.05$, PCOS vs time interaction $P=0.76$), higher AUC_{CP} (370 ± 113 vs 325 ± 104 nmol/180 min $P=0.05$) along with higher levels of GIP (ANOVA PCOS vs CT $P<0.001$; PCOS vs time $P=0.76$) and with higher AUC_{GIP} ($39\,250\pm16\,680$ vs $29\,890\pm7000$ pg/180 min; $P=0.06$). Total active GLP-1 was not higher in PCOS; however, GLP-1 pattern of PCOS was significantly different from CT being lower at 180 min (ANOVA PCOS vs time $P<0.008$).
In conclusion, enhanced insulin secretion in normotolerant women with PCOS is associated to a general augmentation of GIP and to a late-phase decrease of GLP-1. Elevated incretin secretion and the concomitant insulin hypersecretion could be early markers of not yet fully expressed prediabetic state.
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P224

Atorvastatin ameliorates penile erection and sildenafil responsiveness in two distinct animal models of chemical diabetes and inhibits high glucose-induced alterations in human penile smooth muscle cells

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One of the proposed mechanisms responsible for Diabetes mellitus (DM)-associated erectile (ED) dysfunction is overactivity of RhoA/ROCK signalling. Because statins may decrease RhoA activation, we investigated whether atorvastatin ameliorated DM-induced ED in alloxan-treated (100 mg/kg) rabbits (*in vitro* studies) and streptozotocin-treated (STZ, 50 mg/kg) rats (*in vivo* studies). A subgroup of diabetic animals received atorvastatin 5 mg/kg per daily 2 weeks before sacrifice. In diabetic animals, atorvastatin treatment did not affect glycaemia, cholesterol blood levels and the diabetes-induced hypogonadal state. In alloxan-rabbits, atorvastatin did not ameliorate DM-induced acetylcholine hypo-responsiveness, while normalized DM-induced hypersensitivity to the contractile effect of Endothelin-1 and to the relaxant effect of the ROCK inhibitor Y27632. In STZ-rats penile tissue, ROCK1 mRNA resulted increased, nNOS mRNA decreased. These effects were not normalized by atorvastatin, which, in contrast, completely restored the DM-induced hypersensitivity to Y27632, intracavernously injected. Moreover, atorvastatin ameliorated the DM-induced impairment of penile erection obtained by electrostimulation of the cavernous nerve and normalized the sildenafil effect on erectile function, decreased by DM. We then studied *in vitro* effect of atorvastatin on RhoA/ROCK in human foetal penile SM cells (hfPSMCs) activated by increasing concentration of glucose (5, 22 and 40 mM). In hfPSMCs, high-glucose (22 and 40 mM) stimulated RhoA activation, along with migration, proliferation and RhoA-mediated gene transcription. Atorvastatin (1 μM) restored all the high glucose-induced effects, whereas was ineffective in the presence of geranyl–geranyl pyrophosphate, the product reduced by statins and required for RhoA activation. Accordingly, in atorvastatin-treated hfPSMCs RhoA was less expressed at the membrane (active form) and RhoA-dependent genes (ROCK2, myocardin, MKL1, SM22α, desmin, and α-SMA) was down-regulated. In conclusion, atorvastatin, most probably by inhibiting RhoA, is able to decrease the DM-induced ED and also to restore sildenafil responsiveness. This data are in keeping with clinical reports showing that atorvastatin ameliorates sildenafil-induced erections, in otherwise unresponsive subjects.

P225

Circulating endotoxin as a potential biomarker and mediator of inflammation: influenced by diabetic therapies

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Recent studies suggest that endotoxin from gut flora, may be key to development of inflammation by stimulating the secretion of an adverse cytokine profile from adipose tissue. The study investigated the relationship between endotoxin and various metabolic parameters of diabetic patients to determine if anti-diabetic therapies exerted a significant effect on endotoxin levels and adipocytokine profiles of T2DM subjects. Fasting blood samples were collected from consenting Saudi Arabian patients (BMI: $30.2\pm$ (S.D.) 5.6 kg/m^2, $n=413$), consisting of non-diabetics (controls, $n=67$) and T2DM subjects ($n=346$). The diabetics were divided into 5 subgroups based on 1 year treatment regimes: dietcontrol ($n=36$), metformin ($n=141$), rosiglitazone (RSG, $n=22$), a combined fixed dose of metformin/rosiglitazone (Avandamet $n=100$) and insulin ($n=47$). Lipid profiles, fasting serum glucose, insulin, adiponectin, resistin, TNF-α, leptin, C-reactive protein (CRP) and endotoxin concentrations were determined. Regression analyses revealed significant correlations between endotoxin levels and triglycerides ($r^2=0.42$; $P<0.0001$); total cholesterol ($r^2=0.10$; $P<0.001$), glucose ($r^2=0.076$; $P<0.0001$) and insulin ($r^2=0.032$; $P<0.001$). Endotoxin showed strong inverse correlation with HDL-cholesterol ($r^2=0.055$; $P<0.001$). Endotoxin levels were elevated in all of the treated diabetic subgroups compared with control, but only RSG group did not differ significantly from control (Control: 4.2 ± 1.7 EU/ml, RSG: 5.6 ± 2.2 EU/ml). Both Avandamet and RSG treated groups had significantly higher adiponectin levels than all the other groups, with RSG group expressing the highest levels overall. In conclusion, sub-clinical inflammation in T2DM may, in part, be mediated by circulating endotoxin. Furthermore, that whilst the endotoxin and adipocytokine profiles of diabetic patients treated with different therapies were comparable, the RSG group demonstrated significant differences in both adiponectin and endotoxin levels. We confirm an association between endotoxin and serum insulin and triglycerides and an inverse relationship with HDL. Lower endotoxin and higher adiponectin in the groups treated with RSG may be related and indicate another mechanism for the effects of RSG on insulin sensitivity.

P226

Serum adiponectin concentration is not dependent on inflammatory process of periodontium in patients with primary hypertension: preliminary results

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Introduction
Patient with chronic periodontitis (CP), especially those with its advanced forms, have higher cardiovascular risk compared to those with healthy periodontium. They are also characterized by higher hs-CRP levels, greater left ventricular mass, greater intima-media thickness. Adiponectin, a hormone excreted by fat tissue, is involved in insulin sensitivity regulation and in atherosclerotic processes. The aim of the study was to assess a relationship between chronic periodontitis and serum adiponectin concentration in patients with primary hypertension.
Material and methods
Two groups of patients were included into the study: 28 patients with no or only moderate CP (Community Periodontal Index of Treatment Risk–CPITN – score = 0–2, mean age 49.6±5.9 years, BMI 30.6±5.2 kg/m^2), and 25 patients with advanced CP (CPITN score 3–4, mean age 50.9±4.5 years, BMI 28.6± 3.1 kg/m^2). In both groups other than periodontium potential sources of infection were carefully excluded. Serum adiponectin concentration was assessed by EIA (Alpco Diagnostics). Mann–Whitney test was used for between groups comparison, and Spearman rank test for correlation assessment.
Results
Serum adiponectin concentration did not differ between CPITN 0–2 and CPITN 3–4 groups (12.4±5.0 μg/ml vs 12.2±3.9 μg/ml), the same was true for serum hsCRP level (2.99±3.89 mg/ml vs 2.14±2.33 mg/ml, respectively). No significant correlation between adiponectin concentration and mean or maximal CPITN score was shown. There was also no correlation between adiponectin and hsCRP levels.
Conclusion
From those preliminary results one can conclude that there is no relationship between serum adiponectin concentration and inflammatory process of periodontal tissue. However, a small number of included patient and no difference between hs-CRP level between the groups limit power of the study.

P227

The association between adipocytokine profiles and insulin resistance in obese subjects

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This study aims to explore the baseline adipocytokine profiles of adult Saudis and evaluate their relationship in the development of insulin resistance. Three hundred adult Saudis with varying glucose tolerance were recruited. They were grouped into NGT, IGT and DM. Anthropometrics, glucose and lipid profiles were analyzed by routine methods; leptin, adiponectin, resistin and CRP were measured by ELISA. Insulin resistance was significantly correlated with levels of CRP (R 0.32, $P=0.02$) in the NGT; with leptin levels (R 0.46, $P=0.001$) in the IGT; and with adiponectin levels (R 0.25, $P=0.001$) in all groups. In males, resistin and CRP exhibited significant correlations to insulin resistance (R 0.33, $P=0.005$); in females significant correlation was demonstrated between insulin resistance and adiponectin (R 0.32, $P=0.003$). Significant associations exist in the adipocytokine profiles of adults with different glucose tolerance. Certain adipocytokines can be used not only as promising markers but as potential adjunct therapy with regards to insulin sensitivity and obesity.

P228

Vitamin D deficiency is associated with heart failure and sudden cardiac death: cross-sectional and mortality data of 3299 patients referred to coronary angiography

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Background
The myocardium is a target tissue for vitamin D, that was shown to exert several effects on cardiac contractility and calcium homeostasis. We aimed to elucidate whether insufficient vitamin D status is associated with heart failure and sudden cardiac death (SCD).
Methods and Results
We measured 25-hydroxyvitamin D [25(OH)D] in 3299 Caucasian patients that were all routinely referred to coronary angiography at baseline (1997–2000). 25(OH)D was not associated with the prevalence of coronary artery disease (CAD) but was negatively correlated with N-terminal pro-B-type natriuretic peptide (NT-pro-BNP), and was an independent predictor for higher NYHA classes and impaired left ventricular function. During a median follow-up time of 7.75 years, 116 patients died due to heart failure, 90 due to myocardial infarction and 118 due to SCD. After adjustment for cardiovascular risk factors, the Cox proportional hazard ratios (with 95% confidence intervals) for death due to heart failure and for SCD were 2.84 (1.20–6.74) and 5.05 (2.13–11.97), respectively, when comparing patients with severe vitamin D deficiency [25(OH) D < 10 ng/ml)] with the normal range group [25(OH)D ≥ 30 ng/ml]. 25(OH)D was not independently associated with the risk for fatal myocardial infarction. For all statistical analyses we obtained similar results for 25(OH)D and for 1,25-dihydroxyvitamin D [1,25(OH)2D].
Conclusions
Low levels of 25(OH) and 1,25(OH)2D are independently associated with heart failure and SCD but not with fatal myocardial infarction and prevalent CAD, suggesting that possible cardioprotective effects of vitamin D might directly affect the cardiomyocytes and less the coronary vessels.

P229

Retrovirally transduced, antigen-specific T cells for therapy of type 1 diabetes

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In vitro expanded antigen specific CD4+CD25+ regulatory T cells (Tregs), have been shown to suppress autoimmune diabetes, suggesting a novel approach to cellular immunotherapy for autoimmunity. However to interfere with ongoing disease requires at least 10^6 *in vitro* expanded antigen specific Tregs which are difficult to obtain from a polyclonal repertoire. Hence an alternative approach would be to instruct naïve or antigen specific CD4+T cells to obtain regulatory function. Indeed retroviral transduction of antigen specific T cells with*Foxp3*, a Treg lineage specific transcription factor, results in the generation of Tregs that can interfere with established autoimmunity in a non-lymphopenic nonobese diabetic (NOD) mouse model. However, conditions for transduction into NOD T cells have not yet been optimized. Furthermore very little is known about the *in vivo* effect of these *Foxp3* transduced antigen specific T cells. Here we show that ecotropic pseudotyped viruses are more efficient at transducing NOD CD4+T cells than VSVG pseudotyped viruses. In addition retroviral transduction of *Foxp3* into NOD CD4+ primary T cells carrying the transgenic TCR from an islet Ag-specific T cell clone, BDC2.5, confers a Treg phenotype on these cells as they exhibit Treg signature characteristics such as *in vitro* anergy, suppressive capacity and upregulation of CD62L, CD25 and GITR. Adoptive transfer of *Foxp3* transduced BDC T cells into NOD recipients shows that the cells home and proliferate in an antigen specific manner.

P230

Induction of autoantigen-specific tolerance by retroviral transduction of hematopoetic stem cells with GAD65

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Transfer of hematopoetic stem cells from mice transgenically overexpressing the islet autoantigen insulin could prevent type I diabetes in NOD mice. We tested if such an approach of tolerance induction could be employed by means of retroviral gene transfer of autoantigens into syngeneic hematopoetic stem cells (HSCs). We used glutamic acid decarboxylase 65 (GAD) as the autoantigen as T cell responses against GAD are more easily detected in NOD mice and as it has been previously difficult to establish tolerance against this antigen. We generated retroviruses expressing GAD modified for optimal presentation by antigen presenting cells followed by eGFP. Retroviruses expressing just eGFP served a control. We developed a protocol for efficient *in vitro* transduction of NOD-HSCs. Mice reconstituted with GAD transduced bone marrow exhibited a long-lasting, multi-lineage hematopoetic chimerism as shown by eGFP. Percentage of eGFP positivity of HSCs correlated with eGPF expression in thymic and in peripheral dendritic cells. Mice receiving eGFP transduced HSCs showed a strong T cell response against GAD and its peptide epitopes while animals getting GAD transduced HSCs were completely tolerant. Chimerism of just 5% with GAD transduced HSCs was still sufficient to induce complete GAD-specific tolerance. Recipients of secondary bone marrow transplants were also tolerant against GAD thereby proving that tolerance was due to transduction of HSCs rather than of committed progenitors. Despite complete tolerance against GAD mice still developed type I diabetes with unchanged kinetics and incidence as compared to mice receiving eGFP transduced HSCs, thereby proving that GAD65 is not an essential autoantigen in the NOD mouse. We prove for the first time that retroviral transduction of HSCs with autoantigens can lead to complete T cell tolerance. Similar approaches with other autoantigens could have a huge potential in the therapy of type I diabetes. Meanwhile the model will be suitable for testing the importance of potential antigens without the need of creating transgenic animals.

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Cardiac autonomic and peripheral neuropathy in newly diagnosed type 2 diabetic patients

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Background and aims
At diagnosis 20–30% of patients (pts) with type2 diabetes (T2DM) have neuropathy, that often goes unrecognized. Cardiac autonomic neuropathy (CAN) is associated with 5-fold risk of mortality. As today there is no specific treatment for overt neuropathy, it is important to use prevention and timely revealing. The goal of the work was to evaluate prevalence of peripheral neuropathy and CAN in pts with newly diagnosed T2DM.
Materials and methods
We observed 39 pts with newly diagnosed T2DM without known CV disease, ketoacidosis, alcoholism and/or chronic liver disease and non-diabetic nerve damages, not taking medications affecting CAN reflex tests. Ewing's standard reflex tests were performed, severity of CAN was evaluated according to Jermendy, 1995. Standardized evaluation was conducted (Neurometer, Baltimore, MD), that discriminates between neuropathic and non-neuropathic pts and tests different nerve fiber types. Electric current perception threshold was measured at: 5, 250 and 2000 Hz. Measurements were obtained from hallux deep and superficial peroneal nerves. Forced choice method was used to confirm minimum intensity, which was considered as current perception threshold. Clinical data of the study population: males/females-27/12, mean age – 49.3 ± 3.6 years; HbA1c $8.3 \pm 1.73\%$; BMI 24.4 ± 2.6 kg/m^2.
Results
CAN test scores – 0 -1 -18 cases (46.15%), 2–3 (mild) – 17 pts (43.59%) and 4–6 (moderate) – 4 pts (10.26%) CAN. No severe CAN were registered. CPT abnormalities had 14 pts: 8 (20.5%) hyperesthetic and 6 (15.4%) hypoesthetic, 12 of them had CAN. Positive correlation was demonstrated between prevalence of CAN and HbA1c ($P < 0.001$); CAN and peripheral sensory dysfunction ($P < 0.001$).
Conclusion
CAN was observed in 53.85% of the patients; peripheral sensory dysfunction prevalence here was higher (35.9%), than in general population. Undiagnosed hyperglycemia that may be asymptomatic and untreated for years, may explain why at diagnosis CAN and peripheral sensory dysfunction are present in many patients.

P232

Severity of depression in patients with type 1 and type 2 diabetes and its relation to the level of glycemia control and anti-depressive therapy

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Background and aims
There are consistent findings about increased prevalence of depressive disorders in diabetes. The aim of the study was to reveal prevalence of depression in patients (pts) with type 1 (DM1) and type 2 (DM2) diabetes.
Materials and methods
Totally, 157 pts with DM were questioned. To assess depression degree Beck's scale was used, and 83 depressive pts (scores > 10), were allocated into 2 groups (Gr.): Gr.1 ($n = 44$)-DM2 pts (m20/f24), mean age 57.5 ± 8.4 years, diabetes duration 6 ± 2 years, HbA1c-$8.8 \pm 1.3\%$, Gr. 2 ($n = 39$)–DM1 pts (m16/f23), mean age 30 ± 5 years, diabetes duration-7 ± 2 years, HbA1c-$9.2 \pm 1.9\%$. Assessment scores: Gr.1 (m) > 55 (7 pts/35%), 30–50 (8 pts/40%), ≥ 10 (5 pts/25%); (f) > 60 (11 pts/45.8%), 30–50 (8 pts/33.4%), > 15 (5 pts/20.8%). Gr.2(m) > 50 (8 pts/50%), 30–50 (7 pts/43.7%), ≥ 10 (1 pts/6.25%); (f) > 60 (12/52.2%), 30–50 (8/34.8%), > 20 (3/13%). Eighteen Gr.1 pts were treated with insulins and oral hypoglycemic agents (OHAs); 26-only with OHAs. Intensive insulin therapy was used in 22 Gr.1 pts. Tianeptin 3 tablets/daily was used for depression treatment in both groups.
Results
Repeated examination at month 3 post study initiation showed: HbA1c dropped by $7.3 \pm 0.9\%$; $P = 0.000$ (Gr.1), and by $6.5 \pm 0.6\%$; $P = 0.000$ (Gr.2). Beck's scores were: Gr.1 (m): 30–40 (7 pts), 20–30 (8 pts), 0 (5 pts); (f): 50–60 (11 pts), 20–30 (8 pts), ≥ 10 (5 pts). Gr.2 (m): 30–40 (8 pts), ≥ 20 (7 pts), 0 (1 pts); (f): 50–60 (12 pts), 20–25 (8 pts), ≥ 8 (3 pts). At entry there was no statistically evident difference in depression severity between the groups ($P = 0.177$; $P = 0.537$, respectively). Post treatment there was statistically evident correlation between decline in HbA1c levels and depression severity in both groups, this evidence was relevant for males and females: Gr.1 (m) $r = 0.948$, $P = 0.000$; (f) $r = 0.909$, $P = 0.000$. Gr.2 (m) $r = 0.915$, $P = 0.000$; (f) $r = 0.921$, $P = 0.000$.
Conclusion
We conclude: (1) There was no statistically evident difference in depression severity between the DM types. (2) There is statistically evident post-treatment correlation between glycemia control level and depression degree in both groups.

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Quality of diabetes shared care in patients with diabetes type 2 in the diabetes clinic of Fredericia hospital

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Aim
To evaluate the quality of diabetes group-based education followed by shared care. The diabetes education programme had a duration of 4 days and included screening for diabetes late complications. Patients with microvascular complications should visit the diabetes clinic four times a year. Patients without microvascular complications should visit their general practitioner every third month and the diabetes clinic once a year.
Methods
Retrospective data on 100 newly referred patients with type 2 diabetes with quality standards shown in parenthesis.
Results
Eighty-six percent (80%) of the patients visited our diabetes clinic 2 years after the diabetes group-based education and 73% (80%) visited their general practitioner.

After 2 years, HbA1C and blood pressure were assessed in 100% of the patients (>95%), while urinary albumin was measured in 99% (>95%).
Optimal control with HbA1C<7% increased from 36 to 55% (>60%), and moderate good control with HbA1C<8% increased from 75 to 83% (>80%) 2 years after diabetes education.
Optimal control of blood pressure ≤130/80 mmHg was found in 35% (>60%) at the diabetes group-based education and in 40% 2 years after the diabetes group-based education. Blood pressure ≤140/90 mmHg was found in 59% (80%) at the diabetes group-based education and in 62% (>80%) 2 years later.
Micro- and macroalbuminuria was shown in 9% at the diabetes group-based education and 8% 2 years later. Total cholesterol < 4.5 mmol/l in 31% (>80%) at the diabetes group-based education and in 57% (>80%) 2 years later.
Conclusions
The quality of the organization of diabetes care was good with high scores of appearance in the diabetes clinic and measurement of clinical variables. The quality of clinical results was good concerning glycaemic control while, quality goals for the management of blood pressure and cholesterol values were not accomplished.

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Cardiac autonomic neuropathy in relation to some components of metabolic syndrome in newly diagnosed type 2 diabetic patients

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Background and aims
Components of metabolic syndrome (MS) carry an increased risk for cardiovascular disease (CVD). People with abnormal glucose regulation are more prone to develop complications. At diagnosis 20–30% of type 2 diabetic (T2DM) patients (pts) already have neuropathy-one of the most dangerous complications. Cardiac autonomic neuropathy (CAN) is associated with five-fold risk of mortality. Our aim was to study possible relation between CAN and some components of MS in newly diagnosed T2DM pts.
Materials and methods
We observed 33 T2DM pts with Grade 1/2 arterial hypertension (AH) (ESH/ESC Guidelines), increased BMI, but without known CVD, ketoacidosis, alcoholism and/or liver disease. males/females-21/12, mean age-47.5±4.8 years.; mean HbA1c-8.1±2.04%; mean BMI-29.6±3.3 kg/m^2). Ewing's standard reflex tests were performed; severity of CAN was evaluated according to Jermendy *et al.* 1995. Lipid profile, resting blood pressure (BP), pulse pressure (PP) and heart rate (HR) were assessed.
Results
Response CAN reflex tests were normal in 15 cases (45.4%); 13 pts (39.4%) had mild, and 5 (15.1%) – moderate CAN. No severe CAN was registered. There was positive correlation between prevalence of CAN and HbA1c ($P<0.001$); CAN and mean heart rate ($P<0.001$), CAN and mean SBP/PP ($P<0.05$). Only 7 pts had normal lipid profile. High total cholesterol levels (mean 5.59±0.84 mmol/l) were registered in 18 cases, elevated LDL levels (mean 3.86±1.10 mmol/l)-in 4 pts; low HDL levels (mean 0.89±0.69 mmol/l)-in 21 pts; high triglycerides (mean 2.91±0.54 mmol/l)-in 8 pts. No statistically evident correlation was observed between CAN severity and lipid profile indices.
Conclusion
CAN was registered in 54.5% of patients; compared to general population abnormal lipid profile indices were observed more frequently (78.9%) in the study population. We can presume, that in newly diagnosed T2DM hyperglycemia, that may be asymptomatic and not treated for years, plays more important role in CAN development than lipid disorders.

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Cumulative incidence, timing and risk factors for thyroid dysfunction in 144 Romanian patients treated with amiodarone

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Aim
To assess cumulative incidence and risk factors for amiodarone-induced thyroid dysfunction (AITD) in a large cohort of Romanian subjects.
Subjects and methods
One hundred and forty-four patients (59 M/85 F), mean age 61.4±0.9 years, treated 2.4±0.2 years with amiodarone were assessed for TSH, TT_3, TT_4 (immunoradiometric assays) and FT_4, TPO Ab (microenzymatic immunoassays). Thirty-three patients were residents in areas with mild/moderate iodine deficiency and 111 patients from areas with normal iodine intake. Kaplan Meier curves were used for estimation of cumulative incidence and timing of AITD.
Results
AITD occurred in 89/144 patients (61.8%): 60 patients (41.7%) developed thyrotoxicosis (AIT), 29 patients (38.1%) hypothyroidism. Type 1 and mixed forms of AIT prevailed (43 patients, 71.6%). Estimated mean time for AITD was 3.8±0.33 years; estimated cumulative incidence was 25% at 1 year, 50% at 2 years and 75% at 5 years of treatment. Patients from iodine-deficient areas developed thyroid dysfunction significantly more frequent (25% at 0.5 years, 50% at 2 years and 75% at 4 years) and earlier (2.2±0.3 years) as compared to those from iodine sufficient areas (25% at 1.5 years, 50% at 3 years and 75% at 7 years), mean time 4.2±0.4 years, $P=0.001$, log rank Mantel-Cox. Patients with TPO Ab ≥ 100 IU/ml developed thyroid dysfunction significantly more frequent (25% at 0.75 years, 50% at 1 year and 75% at 2.5 years) and earlier (2.2±0.7 years) as compared to those with TPO Ab < 100 IU/ml (25% at 1.1 years, 50% at 3 years and 75% at 5 years), mean time 3.9±0.5 years, $P=0.046$. No differences regarding age, gender, cumulative amiodarone dose were noticed.
Conclusion
Iodine deficiency and thyroid autoimmunity are the main risk factors for amiodarone-induced thyroid dysfunction in Romania.

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Changes in glucose profiles in fasting patients with type 2 diabetes mellitus during Ramadan: analysis of CGMS data in patients in the UAE

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Introduction
Many Muslim patients with diabetes observe dawn to dusk fasting during the month of Ramadan. In an observational prospective study, we have used continuous glucose monitoring system (CGMS) to investigate the changes in glucose profiles amongst Muslim patients during Ramadan fasting.
Methods
CGMS was applied to patients with type 2 diabetes mellitus ($n=14$) attending AlZahra Hospital Sharjah for three consecutive days, before (non-fasting) and then during Ramadan (fasting). Necessary changes to timing and dosing of medication were made. Two CGMS curves were constructed by pooling complete individual 24 h glucose datasets in fasting and non-fasting periods. For each period, area under the curve (AUC), low blood glucose index (LBGI) and high blood glucose index (HBGI) were calculated using methods previously described. For non-diabetic subjects the same procedure and analyses were performed.
Results
Among the diabetic patients studied (3 female, 11 male, age 45.2±7.4 HbA1c 7.8±2.0%, BMI 30.9±4.1 kg/m^2) and also amongst non-diabetic subjects ($n=2$), there were no significant differences in AUC for glucose curves during fasting (1389 mmol/l×5 min daytime, 1354 mmol/l×5 min night-time) and non-fasting (1346 mmol/l×5 min daytime, 1432 mmol/l×5 min night-time) periods. Mean glucose profile during fasting was characterized by a small peak in early morning (corresponding to pre-dawn meal), lower glucose for part of the day and a rapid rise in glucose level with a large peak in the evening (evening meal). LBGI in both fasting and non-fasting periods were below 4. HBGI was higher in the evening during Ramadan.
Conclusion
During Ramadan fasting, in the group studied there was a common problem with hyperglycaemia after the evening meal; hypoglycaemia occurred rarely. Major emphasis on dietary advice and a more appropriate adjustment in antidiabetic treatment could help in achieving better glycaemic control with Ramadan fasting.

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Diabetic ketoacidosis in two periods: XX–XXI centuries

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Despite advances in the management of last years, diabetes and its complications are still the most frequent cause of admission in Endocrine Departments. Nowadays, in our Hospital, 60% of admissions are due to Diabetes, and more than a half of these are diabetic ketoacidosis (DKA) (35.4%).
Objective
Analize DKA episodes in our medical area in the last 12 years and compare them divided in two periods.
Methods
Divide DKA episodes in two periods of 6 years: A: XX century (January 95–December 00) and B: XXI century (January 01–December 06) according to ADA diagnostic criteria. We retrospectively analyzed medical charts.
Results
A (XXc): 117 episodes (56 patients); Type 1 diabetes 95.7%; Mean age: 26.48 ± 15.26 years; Gender: 59% men; Precipitating factors: infections 32.2%, inadequate insulin or diet 28%, drugs 5.1% corticoids 0.8%, unknown cause 33.1%, other 0.8%; DKA- new onset diabetes 12%; evolution of disease (months) : 102.03 ± 81.08; pH: 7.11 ± 0.11; HCO3: 9.79 ± 4.4; Glycemia: 522.62 ± 204.71; Intensive care unit assistance: 6.8%; Admission days: 9.88 ± 6.9.
B (XXIc): 108 episodes (60 patients); Type 1 diabetes 92.6%; Mean age: 31.8 ± 16.25 years; Gender: 61.1% men; precipitating factors: infections 27.1%, inadequate insulin or diet 30.8%, drugs 14%($P=0.02$), corticoids 0.9%, unknown cause 22.4%, other 4.7%; DKA- new onset diabetes 6.5%; evolution of disease (months): 134.68 ± 107.7; pH: 7.10 ± 0.12; HCO3: 9.04 ± 3.94; Glycemia: 569.51 ± 225.54. Intensive care unit assistance: 12%; Admission days: 9.01 ± 8.3.
Conclusions
We observe an increase in drug abuse as a precipitating factor for DKA in the last years, however we haven't demonstrated other differences in clinic and biochemical DKA episodes profile.

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Endothelin-1 and diabetes: influence of cronic metabolic control

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Background
Diabetes mellitus leads to endothelium dysfunction and an accelerated progression of atherosclerosis. Vascular complications of diabetes mellitus can affect not only large and medium arteries resulting in coronary heart disease and peripheral arteries diseases, but also small vessels leading to retinopathy and nephropathy.
Endothelin-1 is mainly synthesized by the vascular endothelial cells and acts on the vascular smooth muscle. Because of its vasoconstrictor and mitogenic effects it plays a role in the development of vascular diseases. In diabetes mellitus atherosclerosis is accelerated. We summarize the available data of the role of endothelin-1 and ANF, in type 1 and type 2 diabetes mellitus and the development of diabetic complication.
Methods and patients
We analysed 21 diabetic patients, 8 woman and 13 men, with ages (X + DS) 61.3 ± 12.6 years and 8.4 ± 9 years of diabetes evolution. Two were diabetic 1 and 19 type 2. The control group were 34 subjects. ET1 and ANF were measured by RIA.
Results
There were no statistically significant differences between ET-1 and Hba1c levels. ($r=0.21$; $P=0.24$).Neither were observated correlation between ANF levels and Hba1c ($r=0.05$; P: 0.79) nor between ANF and ET-1 ($r=0.16$ years $P=0.43$). There were a weak correlation between microvascular disease and ET-1 level (although not statistically significant), but not with ANF levels.
Conclusion
ET-1 and ANF levels not seems to be affected only by metabolic control of diabetes, measured by Hba1c levels. It is possible that other factor could also influence these endothelium dysfunction markers.

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Subclinical alterations of blood pressure associated to clasical cardiovascular risk factors in patients with type 1 diabetes

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Objectives
To evaluate the prevalence of subclinical hypertension detected by ambulatory blood pressure monitoring (ABPM) and its relationship with some clinical and epidemiological factors in normotensive and normoalbuminuric patients with type 1 diabetes.
Patients and methods
Transversal study that included patients with type 1 diabetes, older than 18 years, with clinic blood pressure lower than 130/80 mmHg and absence of microalbuminuria. Twenty-four hours blood pressure monitoring was performed (SPACELABS 90217). We considered normal ABPM those with mean systolic and diastolic blood pressure (sBP and dBP) over 24 h and daytime lower than 130/80 mmHg and over night-time lower than 120/70 mmHg. Subjects with a nocturnal fall in either sBP or dBP of less than 10% of daytime values were classified as non-dippers.
Results
Eighty-five type 1 diabetic patients (55% women) aged 27.9 ± 6.1 years, with a disease duration of 12.3 ± 6.5 years. Mean HbA1c since diagnosis was 8.3 ± 1.4%. In 32% of patients ($n=27$), mean sBP or dBP was altered over daytime and 41.6% of them ($n=36$) were non-dippers. Blood pressure burden was more prevalent in men. BMI was higher in patients with mean sBP elevated over daytime (26.4 ± 3.4 vs 23.5 ± 2.7 kg/m^2, P: 0.002). Non-dippers showed worse metabolic control since diagnosis (HbA1c 8.6 ± 1.4% vs 7.9 ± 1.4%; $P=0.046$). HDL-cholesterol level was lower and triglycerides level higher in subjects with altered sBP or dBP over daytime (HDLc: 55.9 ± 14.4 vs 66.8 ± 13.8 mg/dl, P: 0.003; TG: 97.9 ± 52.5 vs 65.7 ± 13.5 mg/dl, P: 0.003).
Conclusions
(1) Normoalbuminuric and normotensive type 1 diabetic patients present high prevalence of subclinical alterations of blood pressure so ABPM is appropriate to detect them. (2) Burden BP was more frequent in men, in patients with elevated BMI, with worse metabolic control and lipid profile. Association of altered ABMP and classical cardiovascular risk factors suggests that cardiovascular risk factors should be treated soon and intensively, as in type 2 diabetes.

P240

Cognitive dysfunction in diabetics: can we predict it, can we protect them?

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The aim of this work was to estimate chosen cognitive functions in diabetics and to investigate possible correlations between cognitive abilities and chosen clinical and biochemical parameters.
The research was conducted among 122 patients with diabetes type 1 or 2 and 140 clinical healthy people. Cognitive tests such as MMSE test, Clock Drawing Test, Trial Making Test were applied.
In almost 40% of examined diabetics, the significant but mild cognitive impairment was noticed. Cognitive functions impairment and dementia were only observed in people over 50 and were more likely to occure in patients with diabetes type 2. It seems, that diabetes can mostly influence visuo-spatial functions, attention, psychomotor velocity and recent memory. The better results in people with good metabolic control suggest that deficits in cognitive functions are strongly associated with degree of metabolic control such as HbA1c ($P=0.012468$), total cholesterol ($P=0.029565$), HDL ($P=0.022713$) and LDL ($P=0.038056$). The significant impact on cognitive deterioration had a period from stating the diagnosis ($P<0.000001$), presence of chronic complications ($P<0.000001$) and the social and demographic factors like education ($P<0.000001$). Protective effect of the longer period of education, young age and place of living was stated. Cognitive impairments were more likely to occur in patients living in the country but the young age of patients ($P<0.000001$) was a protective factor. The relationship between cognitive function quality and BMI ($P=0.004118$) was confirmed, but it was determined more by the height ($P=0.00019$) than weight of examined patients ($P=0.632958$).
It is supposed that in diabetic patients over 50, there is a need to estimate their cognitive abilities and taking into consideration eventual dysfunctions in the plan of treatment and education.

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Iliofemoral arterial occlusion in type 2 diabetics shortens life duration to the greatest extent: 10 years follow up study

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Background and aim
We have sought to determine factors associated with age at death in patients with type 2 diabetes (T2DM) investigated for peripheral arterial disease (PAOD).
Materials and methods
There were $n=167$ T2DM, 140 with PAOD(G1-4) and $n=27$ Controls; patients were monitored from Jan-1997 till Dec-2006. CW-Doppler examination and segmental pressures showed: 35pts with crural PAOD (G1), 28 popliteocrural (G2), 36 femoropopliteal (G3) and 41 iliofemoral (G4). G1-4 and Controls were comparable in age and diabetes duration.
Results
At the begining there was no difference either in their age or the duration of the diabetes for G1-4 and Controls (65.9 ± 9.2 vs 66.4 ± 9.3, P 0.72; 16.8 ± 8.1 vs 18.2 ± 7.4, P 0.27). However at the end G1-4 died earlier (69.4 ± 8.9) versus Controls (74.1 ± 7.4; P0.045). At the end of this study, only 10 out of 167pts with PAOD (5.9%) and 7 out of the 27 Controls (25.9%) (X^2, $P<0.001$) were alive. Comparison of G1-4 subgroups and Controls concerning age at death: G1 (P 0.85), G2 (P 0.11), G3 (P 0.07), G4 (P 0.01). T-test between G4 ($n=21$ died pts) and Controls (who died, $n=20$): 67 ± 8.4 vs 74.1 ± 8.95, P 0.01, at the time of death; Lec 9.4 ± 2.5 vs $7.3\pm2.3\times10^9$/l, P 0.05; insulin dependens after 12.8 ± 7.7 vs 18.8 ± 7.9 years, P 0.05. X^2 test: male 76 vs 52%, P 0.04; stroke 24% vs 10%, P 0.03. NDS(neuropathy disability scor) >4 86% vs 60%, $P<0.01$; smoking 90% vs 40%, $P<0.01$. In univariate analysis of both G4 and Control groups shorter life expectancy was connected with: HDL ($r=+0.41$, $P<0.01$), tg/HDL ($r=-0.32$, P 0.04), BMI before DM ($r=-0.28$ P 0.04), DM begining ($r=+0.63$, $P<0.01$), IRI (isnulinemia) 9 h ($r=+0.40$, $P<0.01$), age starting insulin therapy ($r=+0.87$, $P<0.01$); Thigh segmental pressure ($r=+0.42$, $P<0.01$), cigarette number ($r=-0.54$, $P<0.01$). In multiple logistic regression analysis statistically significant remaind: shorter DM duration, earlier insulin dependance and number of cigarettes per day.
Conclusion
PAOD, which reduced the life span most significantly was noticed in iliofemoral region; it was characteristic of diabetics whose disease started at earlier age, who were insulin restistant (tg/HDL) and whose pancreas was not as potent (IRI9h). Early introduction of rapid insulins is a very speculative therapeutic option.

P242

Influence of obesity and insulin resistance on scavenger receptor CD36 expression on monocytes in young women

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Introduction
Mechanisms by which visceral obesity and insulin resistance (IR) contribute to atherogenesis are complex and not entirely known. One of the atherogenic factors involved in foam cells formation is scavenger receptor CD36 expressed on monocytes, endothelial cells, adipocytes and platelets, participating in oxidized LDLs absorption and foam cells formation – processes that initiate atheroslerosis. Many factors can influence the expression of receptor CD36.
Aim
To assess the impact of type of obesity and IR on CD36 expression on peripheral blood monocytes.
Materials and methods
The study population consisted of 75 women aged 25–45 years was divided into 3 groups: A1 – 30 obese women with IR, A2 – 30 obese women without IR and control group C – 15 nonobese women. Physical examination included body mass, body mass index (BMI), waist and hip circumferences and waist to hip ratio (WHR) evaluation. CD36 expression was evaluated using flow cytometry. Oral glucose tolerance test (OGTT) with concomitant insulin evaluation was performed. Glucose concentration was measured using enzymatic method and insulin with chemi-fluorescence method. IR (HOMA, FIRI) and insulin sensitivity (QUICKI) indices were estimated.
Results
BMI, WHR, total insulin and total glucose levels during OGTT, HOMA and FIRI were significantly higher in A1 group than in groups A2 and C. In contrast, QUICKI was significantly lower in group A1 than in A2 and C. Expression of CD36 on monocytes did not differ among A1 and A2 groups and was significantly lower in those both than in controls. There were no significant correlations between CD36 expression and BMI, WHR and IR indices.
Conclusions
Expression of scavenger receptor CD36 on monocytes in obese women is significantly lower than in lean individuals. IR indices in obese women show no significant influence on CD36 expression. Obesity significantly reduces CD36 expression and coexisting IR has no infuence on CD36 expression.

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Gestational diabetes has no additional effect on plasma thrombin-activatable fibrinolysis inhibitor antigen levels beyond pregnancy

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Pregnancy is a prothrombotic condition with increased levels of several circulating coagulation factors. The aim of the present study is to investigate the effect of gestational diabetes on plasma thrombin-activatable fibrinolysis inhibitor (TAFI) antigen levels.
Plasma TAFI and PAI-1 antigen levels were measured in 26 pregnant women with gestational diabetes, 25 pregnant women with normal glucose tolerance, and age matched 24 non-pregnant women with no history of gestational diabetes.
Increased plasma TAFI antigen levels were found in pregnant women compared to non-pregnant controls. However, no statistically significant difference in TAFI antigen levels was observed between women with gestational diabetes and pregnant controls. Plasma PAI-1 antigen levels were higher in gestational diabetes than pregnant and non-pregnant controls.
We suggest that pregnancy is associated with enhanced coagulation and impaired fibrinolysis. Despite increased PAI-1 antigen levels associated with gestational diabetes, the effect of gestational diabetes on TAFI antigen levels is lacking.

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Psychological symptoms and quality of life among diabetic patients: a comparative study

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Objective
The aim of this study was to compare psychological symptoms between diabetic patients, asthmatics and healthy individuals and also to determine the effect of these and other variables on quality of life among diabetes patients.
Method
For each respondent the questionnaire on socio-demographic and clinical variables was completed. Each respondent also completed the General Health Questionnaire (GHQ 30), Zung's Self Rating Depression Scale and, the State Trait Anxiety Inventory (STAI 1). All diabetic patients also completed the Diabetic Well-Being Questionnaire.
Results
Depression was more prevalent among diabetic patients (20%) compared to asthmatics (12%) and healthy individuals (4%), while anxiety was more prevalent among asthmatics (34%) compared to diabetics (20%) and healthy individuals (8%). Predictors of depression include early age of onset of diabetes, poor glycaemic control, and the presence of co morbid medical conditions. Factors that correlated significantly with diabetic general well-being include depression, anxiety, duration of diabetes, fasting blood glucose level and presence of co morbid medical conditions. Depression and the presence of co morbid medical conditions significantly predicted a low quality of life.
Conclusion
Psychological factors have significant effects on the diabetic patients' quality of life.

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Correlation between inflammation markers and plasma fasting glucose in overweight and obese women

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Introduction
Elevated levels of the inflammatory markers are associated with increased risk for CVD and diabetes mellitus. There is accumulating evidence indicating that inflammatory markers as C-reactive protein (CRP) is associated with insulin resistance. The aim of the study is to evaluate the correlations between serum inflammatory markers and plasma fasting glucose (PFG) in overweight and obese women.
Methods
Overweight ($25 < BMI < 30$ kg/m^2) and obese (BMI > 30 kg/m^2) 2846 women were enrolled into the study. Anthropometrical parameters were measured. Leukocyte, CRP, ferritin and sedimentation rate were evaluted. Subjects were measured during minimum 12 h fasting period. The subjects were divided into two groups: Group 1 ($n=1982$); women with PFG <100 mg/dl, Group II ($n=864$); women with PFG ≥ 100 mg/dl.
Results
CRP levels were higher with group II than in Group I (6.13 ± 4.34 vs 5.2 ± 3.09 mg/l; $P=0.012$). Serum ferritin levels were higher Group II than in Group I (50.10 ± 46.68 vs 37.51 ± 35.26 ng/ml; $P<0.001$). Leukocyte count and eritrocyte sedimentation rate were higher Group II than group I (7.73 ± 3.71 vs 7.40 ± 1.85/mm^3, $P=0.002$ and 26.67 vs 22.33 mm/h, $P=0.001$). The results are documented on Table.

Table Comparison of inflammation markers within two groups.

Parameter	Group I ($n=1982$)	Group II ($n=864$)	P value
Leukocyte (/mm^3)	7.40 ± 1.85	7.73 ± 3.71	0.002
CRP (ng/ml)	5.20 ± 3.09	6.13 ± 4.34	0.012
Ferritin (ng/ml)	37.51 ± 35.26	50.10 ± 46.68	<0.001
ESR (mm/h)	22.33 ± 12.08	26.67 ± 15.58	0.001

Conclusions
This study shown that increased concentrations of PFG are associated with elevated inflammatory markers in overweight and obese women. Inflammation is closely associated with endothelial dysfunction and cardiovascular risk factors. The Women's Health Study has shown that high CRP levels predict type 2 diabetes. The investigators also have shown that high CRP levels are predictors of the development of type 2 diabetes.

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Phenotypically heterogenous neonatal diabetes within one family caused by a new mutation in the sulphonylurea receptor SUR1 (ABCC8)

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Background
Neonatal diabetes (ND) is a rare, mostly sporadic disorder diagnosed within the first 6 months of life that can either be transient or permanent. We report on a family of four phenotypically heterogenous subjects with ND characterized by a new heterozygous missense mutation (D212I) in exon 5 of the ABCC8 gene encoding the SUR1 subunit of the K_{ATP} channel.
Patients
In each of small-for-gestational-age (SGA) female monocygous twins, ND occurred at the age of 3 months requiring transient insulin therapy for 4 months. At the age of 14 years, clinical diabetes relapsed and insulin treatment had to be restarted. Both twins got one offspring, each. The daughter of the first twin was diagnosed with ND at the age of one month and treated with insulin using continuous subcutaneous insulin infusion (CSII) until 6 months with continuous remission thereafter. Learning disability was diagnosed at the age of 6 years. The son of the second twin was born with SGA and had postpartal hyperglycaemia. CSII was started at day 11 after birth. Furthermore, neurological features with motor and social development delay were present. Molecular genetic analysis was performed reveiling an identical new mutation in all subjects. After molecular diagnosis, treatment of the boy was transferred from insulin (HbA1c 5.8%) to oral sulfonylureas. Fast reduction of insulin and switch of therapy to high-dose glibenclamide was done for the first time completely under ambulatory conditions using real-time continuous glucose monitoring. Metabolic control improved (HbA1c 5.3%) and glycaemic fluctuations as well as frequency of hypoglycaemia decreased significantly. Both mothers were sucessfully transferred to glibenclamide as well.
Summary
In this family, a rare genetic form of neonatal diabetes with neurological development delay and heterogenous genotype-phenotype correlation occurred in combination with a primarily described SUR 1-mutation.

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Continuous subcutaneous insulin infusion with U-500 insulin is better than pramlintide in highly insulin resistant patient with type 2 diabetes

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We describe a 37-year-old woman with a BMI of 32 kg/m^2 who presented with poorly controlled type 2 diabetes (HbA1c of 10.3%). She was requiring 138 units of insulin (Aspart 20, 24 and 30 units pre meal and glargine 64 units at night daily). She previously had diarrhoea with metformin and an allergic reaction to glimepiride. Rosiglitazone was discontinued because of worsening liver function. Her insulin requirement increased in a year to 1650 units daily owing to insulin resistance. She was receiving actrapid 250, 350 and 350 units pre meal and humulin I (changed from glargine) 400 units in the morning and 300 units in the evening. She was commenced on U-500 actrapid insulin to ameliorate the discomfort in sustaining daily insulin injection of 16.5 ml. Subsequently she was tried on pramlintide (symlin) 30 mcg s/c before each meal containing at least 30 g of carbohydrate, while still on insulin. Four months later the patient was on U-500 actrapid 250, 300 and 250 units pre meal and humulin I 250 units in the morning and 200 units in the evening with pramlintide of 180 mcg before each meal (containing at least 30 g of carbohydrate). She lost 5 kg following administration of pramlintide with only slight reduction in her insulin requirement. Her HbA1c was 8.2%. Thereafter she was commenced on CSII (continuous subcutaneous insulin infusion) while still on pramlintide. Later she came off pramlintide and is currently on CSII alone requiring much lesser dose of insulin:U-500 actrapid 1.6 units/h between 0 and 5 h, 1.5 units/h between 5 and 8, 3 units/h between 8 and 9 and 4.7 units/hour at other times of the day (total daily basal dose: 430 units) with a bolus dose of 0.6 units for every 10 g of carbohydrate and with better control of her diabetes(HbA1c 7.5%).

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Systematic exercise can further improve glycaemic control and physical fitness in physically active women with type 2 diabetes

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Background and aims
Exercise intervention programs have been proved beneficial for inactive type 2 diabetes patients by improving physical fitness, anthropometric parameters, glycaemic control and lipid profile. The aim of this study was to examine the additional benefits of a supervised systematic exercise program in physically active postmenopausal women with type 2 diabetes.
Materials and methods
Ten physically active, postmenopausal women with type 2 diabetes under medical treatment (57.4 ± 4.8 years old; 10.6 ± 6.1 years diabetes duration; 3.3 ± 3.0 years of training experience) participated in a supervised systematic program (4 sessions per week for 6 months) combining aerobic (60–80% maximum heart rate) and strength training (70% 1 repetition maximum). Physical fitness, anthropometric and biochemical parameters were measured at baseline and after 6 months of exercise.
Results
Systematic exercise improved physical fitness (VO_2 peak (ml/min per kg): 17.7 ± 3.4 vs 19.9 ± 3.3; power (W): 128.6 ± 30.4 vs 160.7 ± 24.4; muscle strength (kg): upper 26.3 ± 6.9 vs 35.6 ± 4.2, lower 26.9 ± 8.0 vs 35.6 ± 5.0; $P<0.05$). Exercise training decreased HbA_{1c} (7.6 ± 0.5 vs 6.7 ± 0.8%; $P<0.05$) and triglycerides (124.7 ± 44.8 vs 92.6 ± 29.9 mg/dl) whereas there was no change on total cholesterol, HDL, LDL and fibrinogen. Furthermore, body mass was reduced (81.5 ± 8.6 vs 79.0 ± 9.7 kg; $P<0.05$), as well as waist circumference (102.8 ± 11.8 vs 99.8 ± 11.1 cm; $P<0.05$).
Conclusion
Supervised systematic exercise combining both aerobic and strength training significantly improves oxygen consumption, power and muscle strength, has

additional benefits on glycaemic control, body composition and lipid profile and therefore ameliorates the health status of physically active women with type 2 diabetes.

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Early major complications at diabetics in coronary care unit

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Early and late complications of acute myocardial infarction are common at diabetics compared to nondiabetics. The blood sugar level can be one of relevant factors to determine the outcome of patients with acute coronary syndrome.
The aim of our study is to determine the early hospital mortality at diabetics experiencing an acute coronary syndrome.
The data were collected in the coronary care unit of our Institution, for the subgroup of diabetic patients presenting with an acute coronary syndrome as an inclusion criteria. During analyzed period the total number of patients diagnosed as acute myocardial infarction was 465, male/female ratio was 2.72, with the most presented group age of the analyzed 56–65 years old. The early death during first days of hospitalization for an acute coronary syndrome occurred in 56 (12.04%) patients, 12.5% of whom were diabetics.
Diabetes mellitus is not only a risk factor of coronary heart disease but a very important one in determining the occurrence of fatal complications at acute coronary syndromes.

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Nebivolol in diabetic patients with chronic heart failure

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Background and aims
The aim of this study was to study the effect of Nebivolol on clinical course and heart rate variability (HRV) parameters in type 2 diabetes (T2DM) patients (pts) with coronary heart disease (CHD) and Chronic heart failure (CHF) of III-IV FC.
Materials and methods
Participants were 35 T2DM pts with CHD and CHF/ III-IV FC. The pts were divided into 2 groups (Gr.) Gr.1 ($n=15$, 10m/5f, mean age 59 ± 7 years) on basic therapy. Gr.2 ($n=20$, 13m/7f, mean age 61 ± 4.5 years) – Nebivolol was added to the basic therapy. Treatment was initiated with 2.5 mg/daily, dose increased to 5 mg/daily. Nebivolol was administered for 3 months. Echocardiography, to study end-diastolic diameter of LV, EF and FS and Holter ECG to assess myocardial ischemia (MI) episodes, HRV and arrhythmia were performed.
Results
In both groups treatment resulted in improvements in CHF course, reflected by decrease in FC of CHF. EDV evidently dropped in both groups, though for Gr.2 it was more pronounced, than for Gr.1 (20.3% vs 17.3%) EF and FS increased by 21.3% and 23.8%, respectively (Gr.1) and 26.1 and 29.9% respectively (Gr.2). The use of Nebivolol resulted in improvement of HRV parameters – SDNN ($P=0.000$) and triangular index ($P=0.000$). HRV parameter improvement was significant in Gr.2 when compares to Gr.1. SMI episodes also became less frequent in Nebivolol group. Post-treatment complex ventricular arrhythmias were observed more frequently in Gr.1. than Gr.2 (33.3 vs 20%).
Conclusion
Nebivolol in T2DM pts with CHD and CHF positively effects the clinical course of CHF, LV systolic function and ventricular rhythm impairment, decreases MI, increases HRV parameters. Nebivolol does not deteriorate lipid and carbohydrate metabolism makes it possible to successfully use it in T2DM pts with CHD and CHF.

P251

Frequency of hypertension studied by ambulatory blood pressure monitoring in type two diabetes

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Objectives
To know the frequency of hypertension in a group of males with type two diabetes without the antecedent of hypertension, by means of ambulatory blood pressure monitoring (ABPM). To assess the possible differences between the subjects with and without hypertension in age, time of duration of diabetes, obesity and the features of metabolic syndrome.
Methods
We included consecutively 55 males with type two diabetes without the antecedent of hypertension. We made an anthropometric and analytic evaluation and an ABPM during 24 h. For the diagnostic of hypertension we used the 'VII Report of the Joint National Committee on prevention, detection, evaluation and treatment of high blood pressure'. For the diagnosis of metabolic syndrome we used the 'NCEP ATP III guidelines'.
Results
We detected high blood pressure in 20 patients (36.4%). The most part (50%) had elevated systolic and diastolic rates, 35% only systolic and 15% diastolic. The blood pressure reduction during sleep was different between the groups with and without hypertension: dipper (62 vs 20%), non dipper (60 vs 32%) and riser (20 vs 6%), χ^2 $P=0.01$. The patients with hypertension had metabolic syndrome in 90% vs 48% of the patients without it (χ^2 $P<0.01$).
Conclusions
One third of diabetic patients without antecedent of hypertension had it, discovered with ABPM. The most common was the increment of both systolic and diastolic. The blood pressure reduction during sleep was pathologic in 80% of patients with hypertension and 33% without it. The most part of the patients with high blood pressure had metabolic syndrome.

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Concentrations of steroid hormones different from estradiol may constitute risk factors of coronary artery disease in women

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Abstract
The aim of our research was to find different from estradiol steroid hormones that changes in their concentrations may be risk factors of coronary artery disease (CAD) in women.
Material and methods
YM-CAD-38 menstruating women in the age of 42.6 ± 3.4 years with past myocardial infarction and angiographically proven arteriosclerosis. YM-H- 15 healthy menstruating women in the age of 41.1 ± 3.5 years. PW-CAD- 26 postmenopausal women in the age 59 ± 7 years with angiographically proven CAD. 75% of them suffered from myocardial infraction. PW-H- 17 healthy postmenopausal women in the age 66 ± 9 years. From all of the women venous blood samples were taken ones at 0800, in 4–7 day of menstrual cycle from women in the reproductive age. Using immunological methods blood concentrations of estradiol, testosterone, dehydroepiandrosterone sulphate (DHEAS), follicle-stimulating hormone, luteinizing hormone, progesterone and cortisol were measured.
Concentration of the hormones were compared between YM-CAD and YM-H and between PW-CAD and PW-H. Logistic regression analysis (LRA) was applied to find a relation among concentrations of the hormones and occurrence of CAD.
Results
Testosterone was the only hormone of significantly different concentration between YM-CAD and YM-H (3.5 ± 1.5 vs 2.4 ± 1.0 nmol/l, $P<0.014$).
There was a trend in PW-CAD to higher than in PW-H concentration of cortisol (497 ± 138 vs 414 ± 166 nmol/l, $P<0.07$) and lower concentration of DHEAS (2.04 ± 2.07 vs 2.77 ± 2.19 μmol/l, $P<0.08$).
In LRA relation to occurance of CAD was revealed for level of testosterone >3.7 nmol/l in young women (OR 2.5, $P<0.021$) and for concentration of DHEAS <2.58 μmol/l in postmenopausal ones (OR 4.8, $P<0.027$).
Conclusion
Elevated concentration of testosterone in young women and diminished level of DHEAS in postmenopausal women may constitute risk factors of CAD.

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The stress-related protein p8 protects beta cells from streptozotocin (STZ)-induced cell death *in vitro*

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Within the exocrine pancreas, p8 expression is induced by pancreatitis and protects exocrine tissue from inflammatory damage via inhibition of NFκB. In previous work, we investigated the role of p8 within the endocrine pancreas and characterised p8 as a glucose-dependent mediator of beta cell proliferation. Here we investigate the hypothesis that p8 protects beta cells from cell damage by the diabetogenic drug STZ. We investigated native and transiently transfected INS-1E and beta-TC3 beta cells. For transfections, we used a CMV-driven p8 expression plasmid or an empty plasmid control (mock). STZ-induced p8 gene expression and viability of cells were established with a MTS assay and qPCR using commercially bench-tested primers, respectively. Initially we characterised time and dose response of cell lines to 6, 12, and 24 h STZ. Mouse beta-TC3 (24 h LD50, 5 mM) were substantially more resistant to STZ than rat INS-1E (24 h LD50, 0.7 mM). In both cell lines, 6 h exposure to STZ dose-dependently enhances endogenous p8 gene expression to a maximum of about 4-fold at 24 h LD50 concentrations. Further increase of STZ dosages continuously reduces p8 gene expression indicating that endogenous p8 cannot be induced in lethally damaged beta cells. Furthermore, transient p8 overexpression enhances cell viability after 24 h exposure to STZ below 24 h LD50 dosages in both INS-1E and beta-TC3 about 20% despite only low efficiency of liposomal gene transfer (INS-1E, 30–50%; beta-TC3, 10–30%). The protective effect of ectopic p8 expression is abolished at dosages higher than 24 h LD50 indicating that enhanced p8 levels cannot protect lethally damaged cells. In conclusion, these results demonstrate that, in beta cells, p8 is a stress-induced factor, which exerts protection against STZ-induced cell death. Whether p8 mediates its protective effects only via stimulation of cell proliferation or also via inhibition of apoptosis is under current research.

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Increased serum AGEs is a distinct finding in lean women with PCOS

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In insulin resistant, young women with PCOS elevated serum AGEs and their receptor RAGE have been reported. The present study (approved by the local ethical committee) was undertaken to determine whether increased levels of serum AGEs in PCOS is a distinct finding compared with age- and BMI-matched women, presenting the isolated components of the syndrome and whether serum AGE levels were different among PCOS phenotypes. A total of 193 lean non-insulin resistant women were studied. 100 women diagnosed as PCOS (Rotterdam criteria), and further divided to quartiles of well-defined phenotypes. 68 women matched for age and BMI with the isolated components of the PCOS phenotype (HYPER = biochemical hyperandrogenemia only, $n=25$; ANOV = anovulation only, $n=21$ and PCO = US-PCO morphology only, $n=22$) were also studied along with 25 women, who served as controls. Serum AGE levels as well as the metabolic, hormonal profiles and intravaginal ultrasound were determined in all subjects. PCOS population phenotypes and controls did not differ in BMI ($P=0.152$), waist-to-hip ratio (WHR; $P=0.495$), fasting insulin concentration ($P=0.655$) and glucose-to-insulin ratio (GIR; $P=0.320$). Total PCOS women (exhibited statistically higher AGEs levels (7.96 ± 1.87 U/ml, $P<0.001$) compared with women with isolated hyperandrogenemia (5.61 ± 0.61 U/ml), anovulation (5.53 ± 1.06 U/ml) and US-PCO (5.26 ± 0.25 U/ml) as well as with the controls (5.86 ± 0.89 U/ml). The present study shows, for the first time, that women with PCOS had statistically significant elevated serum AGE levels compared with women with isolated hyperandrogenia, anovulation and US-PCO. No difference was detected in AGEs levels among the different PCOS phenotypes. The above data suggest that serum AGEs is a distinct finding characterizing only women suffering from PCOS.

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Importance of vasopressin and aldosterone for clinical outcome of patients after cardiac arrest

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Aim
Assessment of possible role of arginine vasopressin (AVP) and aldosterone (Ald), hormones managing blood pressure and water-electrolyte balance, for clinical state and survival of patents after cardiac arrest (CA).
Material and methods
Fifty-two patients after CA in the age 62 ± 13 years, 29 patients after out-of-hospital CA, 23 after in-hospital CA. Twenty-eight patients died after CA (CA-D), 24 survived (CA-S). In each day after CA clinical state of the patients was assessed by common scales applied in intensive care: GCS, APACHE II, SAPS II and MODS. Just after CA and in 2 following days at 8.00 a.m. blood venous samples were taken from each patient to measure typical laboratory parameters and concentration of AVP and Ald.
Results
Mean concentration of AVP was higher in CA-D than in CA-S in each day after CA. Significant correlations were found among concentrations of AVP in first two days after CA and values of the scales. In CA-D, compared to CA-S, concentration of Ald was higher in each day, significantly in the 3rd one after CA (500 ± 507 vs 235 ± 230 pmol/l, $P<0.04$). Significant relation to fatal outcome was found for concentration of AVP > 83 pmol/l, assessed just after CA, in logistic regression analysis (LRA) (OR = 0.23 for survival, $P<0.01$) and in Kaplan–Meyer survival analysis (SurA) ($P<0.02$), and for concentration of Ald > 222 pmol/l, measured in the 3rd day after CA, in LRA (OR = 0.18 for survival, $P<0.02$) and in SurA ($P<0.04$).
Conclusions
1-AVP is involved in protecting survival in the early stage after CA, Ald plays important role in mechanisms responsible for survival in further stages after CA. 2-High concentration of AVP just after CA and markedly elevated concentration of Ald in the 3rd day after CA are markers of bad prognosis.

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Concentration of interleukins as prognostic factor in patients after cardiac arrest

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The aim of the research was to assess whether concentrations of inflammatory markers in blood of patients after cardiac arrest (CA) are related to clinical state and survival.
Material and methods
Forty-six patients after CA, 21 after out-of-hospital and 25 after in-hospital CA, in the age 63 ± 12 years, were enrolled. Clinical state of them was evaluated by means of Glasgow Coma Scale (GCS) and Acute Physiology and Chronic Health Evaluation II (APACHE II). In the day next to the day of CA (day-1) and in the following day (day-2) were measured blood concentrations of high specific C-reactive protein (hs-CRP), tumor necrosis factor TNF-alfa, interleukin10 (Ile-10) and interleukin-6 (Ile-6). 25 patients survived after CA and were discharged from hospital (CA-S), 21 died during hospitalization (CA-D).
Results
In CA-D patients, compared with CA-S, we found significantly higher concentrations of hs-CRP (in day-1 19 ± 5 vs 15 ± 4, in day-2 21 ± 3 vs 16 ± 5 mg/l, $P<0{,}001$) and Ile-6 (in day-1 24.9 ± 19.8 vs 9.2 ± 11.3, in day-2

24.2±19.7 vs 6.9±6.8 IU/ml, $P<0{,}001$). Concentrations of TNF-alfa were greater in CA-D than in CA-S in day-1 (0.42±0.75 vs 0.18±0.21 IU/ml, $P<0.04$).
Concentrations of hs-CRP and Ile-6 were correlated with values of GCS and APACHE II. Using logistic regression analysis significant relation of concentrations of hs-CRP and Ile-6 in day-1 and in day-2 with survival after CA was proven.
Conclusion
Early strong immunoinflammatory reaction, expressed in higher concentrations of Ile-6, hs-CRP or TNF-alfa, is correlated not only with worse clinical state of patients after CA but with diminished survival of them, as well.

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Cytokines secretion in long-standing diabetes mellitus type 1 and 2: associations with low-grae systemic inflammation

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Background
Low-grade systemic chronic inflammation has been recognized in diabetes. The purpose of this study was the assessment of proinflammatory cytokines secretion profile in long-standing diabetes along with the presence of systemic inflammation.
Methods
Twenty patients with type 1 (T1DM: 7 women, age (±S.E.M.): 30.90±1.93 years, duration of disease: 142.47±28.34 months) and 21 patients with type 2 diabetes mellitus T2DM: 11 women, age: 58.67±3.01 years, duration of disease: 68.32±17.36 months) were studied along with 34 healthy subjects (17 women, age: 53.61±2.48 years). Metabolic parameters were assessed. Serum levels of high sensitivity C-reactive protein, interleukin-1β, interleukin-6 and tumor necrosis factor-α were determined by ELISA. The number of cytokine-secreting peripheral blood mononuclear cells before and after mitogenic stimulation was determined by the Enzyme-Linked-Immuno-spot assay.
Results
Patients with T2DM had an adverse metabolic profile compared to patients with T1DM and controls. There was a difference in hsCRP, sIL-6 and TNF-α level between groups, with higher levels being observed in T2DM and lower levels in controls. T2DM patients were characterized by higher count of cytokine-secreting PBMCs compared to patients with T1DM and controls. Patients with T1DM showed a higher count of IL-1β-secreting PBMCs compared to controls. After stimulation with PMA, an increased number of all cytokine-secreting PBMCs were observed in the studied groups, but this rise was higher in the control group compared to diabetic groups.
Conclusion
The present study revealed increased levels of several circulating markers of chronic inflammation in diabetic groups compared to healthy subjects, but the grade of inflammation was higher in T2DM. Furthermore, both groups presented a defect in cytokines production by the PBMCs after stimulation compared to healthy subjects. The presence of low grade inflammation and the abnormal PBMCs function in diabetic patients may result in the long-term sequelae of diabetes such as the accelerated atherosclerotic process and the susceptibility to infections.

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Rapid glucose spray™ for the management of hypoglycaemia in children with type 1 diabetes

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Hypoglycaemia continues to be a difficult condition to treat especially in infants and young children who cannot administer treatment themselves. Administering the correct dose of sugar is difficult for several reasons: the child may not want to intake food and it may be difficult to force the child to chew or swallow, it is difficult to identify the quantity of sugar necessary to reach optimal glucose levels. If too much sugar is administered, high levels of blood glucose may result from liver glycogenolysis and, to reduce hyperglycaemia, the patient needs more insulin. However if more insulin is administered the child will need more food. This situation produces a 'vicious circle' and an alternative therapy is needed.
The aim of the study was to evaluate the effect of administering small amounts of glucose through the glucose RapidSpray™ (GRS) during hypoglycaemia to prevent hyperglycaemic rebound in children with type 1 diabetes (T1D) and to study the efficacy of RGS so as to improve the metabolic control.
We designed an open randomized trial in children with T1D aged 1–5 years.
Forty-eight patients with T1D, age-group 1–5 years, were randomly allocated into 2 groups: (A) RGS in the treatment of early warning signs of hypoglycaemia (10–20 puffs of RGS, 0.5–1 g glucose) during episodes of early symptoms of hypoglycaemia; (B) traditional treatment of hypoglycaemia episodes.
Parameters of efficacy were: (1) HbA1c at time 0, at 3 months follow-up and at the end of the study, (2) features of hypoglycaemia episodes, (3) quality of life.
At present 17 patients have been introduced in the study. No adverse effects were observed and treatment with RGS was easily accepted by children and mothers. Quality of life under the unpleasant circumstances of hypoglycaemia can be improved by the use of RGS.

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Obestatin inhibits high glucose-induced reactive oxygen species production and apoptosis in bovine aortic endothelial cells

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Endothelial dysfunction is thought to be a major cause of vascular complications in diabetes. Our research shows that obestatin, a 23-aminoacid amidated peptide recently identified as a product of the ghrelin gene, inhibited high glucose-induced apoptosis in cultured bovine aortic endothelial cells (BAEC). Exposure to high glucose concentration (30 mM) for 72 h caused a significant increase in apoptosis, as evaluated by Hoechst staining, but co-treatment of rat obestatin (from 10^{-11} to 10^{-7} nM) eliminated in a dose–dependent manner high glucose-induced apoptosis in BAEC. Obestatin also protected endothelial cells from high glucose by reducing reactive oxygen species (ROS) production. Blockade of adenylyl cyclase and cAMP-dependent protein kinase A signalling prevented the inhibitory effect of obestatin on ROS production. Obestatin also activated phosphatidyl inositol 3-kinase (PI3K/Akt) and ERK1/2 pathway, whereas PI3K and ERK inhibitors counteracted the obestatin anti-apoptotic effect. Finally, saturation binding studies with radioiodinated [^{125}I]-obestatin recognized high-affinity ($K_d=0.5$ nM) specific binding sites in the BAEC cell line, suggesting that these sites may be involved in the cytoprotective effect of the peptide. In conclusion, the results of our study demonstrate, for the first time, that obestatin inhibits both high glucose-induced apoptosis and ROS production in endothelial cells and suggest that this peptide may have potential in preventing vascular complications in diabetic patients.

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The high prevalence of testosterone deficiency syndrome and erectile dysfunctions in the aging men with diabetes mellitus type 2

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Objective
The aim of the study was to determine the prevalence of subclinical late-onset hypogonadism (SLOH) and erectile dysfunctions in the elderly men with diabetes type 2.
Material and methods
We investigated 51 men mean aged 68.3 years with diabetes type 2 according ADA recommendations. Androgens concentrations, BMI, HbA1C were measured. All of men were treated with oral antidiabetic drugs or insulin. LOH was expected on the basis of low testosterone concentration ($\leq$3.5 μg/l) and the

index T/LH ≤ 1, while erectile dysfunctions were expected according IIEF score.
Results
The mean duration of diabetes was 18.4 years. The mean testosterone concentration was 4.09 ± 1.4 ng/ml and the mean T/LH index was 0.82 ± 0.29. Seventy-five percent of men presented the criteria of SLOH according the T/LH index but only 44% had testosterone levels below 3.5 ng/ml. About 65% patients had IIEF score below 16 points. There was inverted correlation between the T/LH index and duration of diabetes, BMI and age ($P < 0.02$). The mean DHEA-S levels also negative correlated with duration of diabetes and age ($P < 0.05$), but not with BMI. There was also inverted correlations between testosterone levels and T/LH index and IIEF score ($P < 0.005$ and $P < 0.002$ respectively).
Conclusions
In elderly men with diabetes mellitus 2 the prevalence of SLOH is about 75%, while ED is about 65% and were more common than in population of elderly men without diabetes. The T/LH index was more sensitive in these men as the criteria of diagnosis of SLOH and ED than total testosterone concentrations. In all the patients with diabetes mellitus the possibility of SLOH and ED should be investigated. In the other hand, elderly men with SLOH also must be screening to exclude the symptoms of diabetes and erectile dysfucntions.

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The influence of testosteone replacement therapy on erectile dysfunctions in aging men with testosterone deficiency and diabetes mellitus type 2

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Objective
The role of testosterone in the anatomy and physiology of erection is becoming clear. Erectile dysfunctions (ED) are very common observed in men with diabetes mellitus type 2 (DM2). The aim of the study was to determine the efficacy of testosterone adminisartions in elderly men with ED and DM2.
Material and methods
We investigated 41 men mean aged 65.4 years with late onset hypogonadism according Polish Endocrine Society recommendations and DM2 according ADA recommendations, who had not been treated with PDE5 inhibitors. All men received testosterone enanthate in dose of 200 mg. i.m. every second week. Durations of treatment was mean 12 weeks. Efficacy of treatment was measured by the IIEF domain before and after 4 and 12 weeks of treatment. All of men were treated with oral antidiabetic drugs or insulin. Hypogonadism was expected on the basis of low testosterone concentration (≤ 3.5 μg/l) and the index T/LH ≤ 1.
Results
After 4 weeks of treatment 21/41 patients showed improvement in erections. At the and of 4 month 32/41 patients showed improvement in the score on IIEF (from 7.9 ± 3.2 to 12.3 ± 3.4; $P < 0.05$). There were inverted correlations between testosterone levels and efficacy of testosterone treatment and also between duration of DM2 and IIEF score.
Conclusions
In elderly men with hypogonadism related to aging (late-onset hypogonadism) and diabetes mellitus 2 after 1 month of testosterone replacement therapy there was a small number of patients in whom treatment results in erectile improvement, but many more patients benefit after 3 months of therapy. Testosterone therapy is time-dependent and combination with PDE5 inhibitors should start only after at least 3-months of monotherapy with testosterone.

Endocrine disruptors

P262

Lipid peroxidation, antioxidant enzymes activities in testis and spermatotoxicity of rats during short-term exposure to atrazine

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Atrazine is a chloro-s-triazine herbicide that has been in used worldwide for over 4 decades now. Its endocrine disrupting effects have been shown in mammals but the specific mechanism or mechanisms of action remain unknown. The aim of this study was to evaluate the acute effects of atrazine on testicular antioxidant systems and on some spermatological parameters in rats.
Atrazine was administered to wistar rats at a dose equivalent to 120 mg/kg or 200 mg/kg b. wt daily for seven days. The results indicate a decrease in the terminal body weight and food consumption in both treated groups. Testicular and epididymal sperm number, spermatozoa viability and motility in atrazine treated animals decreased significantly. Therefore atrazine treatment provoked a significant decrease in daily spermatozoal production. The induction of abnormal sperms was increased by atrazine. These effects were seen in a dose dependent manner. While there were no significant effects on lipid peroxidation, superoxide dismutase (SOD) and catalase (CAT) activities and H_2O_2-generation in the atrazine groups, glutathione (GSH) and glutathione-*S*-transferase (GST) activities in the testis after the last day of treatment showed a significant increase vs control. The epithelium of the seminiferous tubules showed normal histology.
Possibly the dose and duration of exposure was inadequate to induce tissue pathology or that atrazine have a direct effect on spermatozoa. Our study has added the information that oral atrazine exposure attributes to alterations in spermatological parameters without significant effects on the parameters of testicular oxidative stress. This appears not to be mediated by the seminiferous tubules epithelium and may be secondarily related to the restricted food intake and growth rate. Furthermore, our data implicates the GSH/GST status as sensitive markers for atrazine testicular toxicity during short-term exposure.

P263

Endocrine disruption in lower vertebrates: Xenobiotic exposure affects testicular structure and biochemical composition of semen in a male teleost

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Natural and synthetic chemicals such as estrogenic and androgenic compounds have been observed to contribute to the pool of endocrine disruptors in the effluent water from sewage treatment works and may interfere with the endogenous hormonal system regulating reproduction in aquatic organisms. The teleost *Zoarces viviparus* is a recognized model used in aquatic toxicology due to the existence of a maternal-fetal trophic relationship with embryos/larvae developing in the ovary during a period of approx. 5 months.
In the present work *Z. viviparus* males were exposed in a flow through seawater system to different xenoestrogens and the effect on testicular structure, seminal fluid volume, spermatocrit and Sertoli cells was studied.
The xenoestrogenic exposure was observed to severely affect gonadosomatic index. and testis histology. The testes appeared degenerated with cellular abnormalities, increased fibrosis in the interstitium between the seminiferous lobules and with abnormal Sertoli cells phagocytising spermatozoa and spermatids. The normal composition of semen was also affected as observed by a decrease in seminal fluid volume and amino acids and a concomitant increase in spermatocrit, Ca, K, and Mg ions.

P264

Investigating the effects of Liquorice consumption on Salivary Steroid hormones profile and blood pressure in healthy volunteers

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Glycyrrhetinic acid (GA) has diverse *in vitro* effects as an inhibitor of 11beta hydroxysteroid dehydrogenase (11HSD), 5alpha reductase and hormone receptor binding. However, *in vivo* GA studies have focussed on the hypertensive effects associated with the syndrome of apparent mineralocorticoid excess (SAME) in which 11HSD inhibition allows glucocorticoid hormones to bind inappropriately to MR and subsequent decreased aldosterone synthesis. Here we consider whether GA's other *in vitro* effects are reflected in altered steroid hormone profiles *in vivo*.
The effect of liquorice (containing GA) has been assessed by measuring steroid hormone levels in healthy individuals. Ten men and 10 women (18–30 years) were given 100 g liquorice sweets (3% liquorice extract) or non-liquorice containing confectionary for 7 days in a crossover study. Saliva was collected 30 min after waking, at 1100–1300 h and at 1800–2100 h for cortisol, cortisone,

aldosterone (Aldo), deoxycorticosterone (DOC), DHEA and testosterone measurements by in-house, sensitive and specific ELISA methods. Systolic blood pressure measured at the end of the two periods of treatment showed non-significant increases ($P=0.127$) in response to liquorice consumption. Cortisol was significantly higher ($P=0.003$) and cortisone and aldo were reduced by liquorice ($P<0.001$) consistent with the SAME. DHEA, testosterone and DOC were increased ($P<0.001$) possibly because of reduced hepatic clearance. However, these steroids are not 11HSD type 2 substrates indicating liquorice might also have inhibited hepatic 5alpha reductase. Recent studies also implicate the bi-directional 11HSD type1 enzyme in DHEA metabolism. GA is equipotent as an inhibitor of 11HSD type 1 and type 2 oxidase activity. 7-Hydroxy-DHEA, a major metabolite of DHEA is inter-converted with 7-keto-DHEA by 11HSD type 1 and could, therefore, secondarily interfere with DHEA metabolism. We conclude that in addition to cortisol, other biologically active steroid hormones are affected by liquorice. Increased salivary levels of DHEA and testosterone suggest that liquorice modulates their bioavailability.

P265

Clinical and laboratory symptoms of metabolic syndrome in women in climacteric period

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The aim of the study was the analysis of dependencies between sex hormones and metabolic syndrome.
Forty-five women with metabolic syndrome and 49 healthy women were examined.
The diagnostic criteria of metabolic syndrome: waistline $\geq$80 cm, waistline/hips index WHI$\geq$0.85 and also having 1 or 2 cardiovascular risk factors: glucose$\geq$100 mg%, RR$\geq$130/80, HDL$<$ 50mg% and TG$\geq$150 mg%.
The levels of estradiol (E_2), testosterone (T), dehydroepiandrosterone sulphate (DHEA-S), sex hormone binding globulin (SHBG) were measured. Free testosterone index (FTI), free estradiol index (FE_2I) and free testosterone (FT) were determined by means of a calculation method.
A significant difference between the group with metabolic syndrome (FTI-5.13$\pm$3.72, FE_2I-517.28$\pm$539.69, SHBG-48.49$\pm$32.18 nmol/l) and the control group (FTI-3.04$\pm$1.65, FE_2I-281.72$\pm$313.6, CRP-1.49$\pm$1.38 mg/dl, SHBG-61.28$\pm$30.89 nmol/l) concerned FTI($P<0.01$), FE_2I ($P<0.05$), SHBG ($P<0.05$).
There was a significant correlation of waistline with SHBG ($r=-0.274$), with FTI ($r=0.324$) and with FE_2I ($r=0.248$) and WHI with SHBG ($r=-0.239$), with FTI ($r=0.302$), with FE_2I ($r=0.210$) in women with metabolic syndrome.
The pathogenesis of metabolic syndrome in menopausal women is connected with bioavailability of sex hormones and binding proteins.

P266

Functioning of endothelium in metabolic syndrome in the climacteric period of women

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The aim of the work was the analysis of dependencies between sex hormones and functioning of endothelium in women in the climacteric period with metabolic syndrome.
Fifty-three women with metabolic syndrome and 45 healthy women were examined.
Lipidogram (LDL: cholesterol-LDL, HDL: cholesterol-HDL), triglycerides(TG) and fasting glucose levels were determined. Body mass index BMI and waistline/hips index (WHI) were calculated.
The levels of testosterone (T) were measured using the RIA method. The level of sex hormone binding globulin (SHBG) in blood was calculated using the IRMA method and CRP using the turbidymetric method. The levels of matrix metalloproteinases (MMPs-9), tissue inhibitors of metalloproteinases (MMP-1) and human tumor necrosis factor alpha (TNFα) were measured using immunoassay method.
Free testosterone index (FTI) and free testosterone (FT) were determined by means of a calculation method. Fasting plasma glucose level was determined by enzymatic method with glucose oxidase.
The mean levels of CRP in groups with metabolic syndrome were 2.5$\pm$2.12 mg/dl, in healthy women 1.64$\pm$1.56 mg/dl ($P<0.05$).
There was a positive correlation ($P<0.05$) between TNFα: with FAI ($r=0.233$, $P<0.05$, $N=69$), with the percentage of free testosterone ($r=0.328$, $P<0.05$, $N=69$), with percentage of bioavailable testosterone ($r=0.329$, $P<0.05$) and negative correlation with SHBG($r=-0.236$, $N=69$).
There was asignificantly positive correlation of TIMP1: with BMI ($r=0.308$, $P<0.05$, $N=63$), with waistline ($r=0.372$, $P<0.01$, $N=63$), with WHI ($r=0.288$, $P<0.05$, $N=63$).
There was a significantly positive correlation of MMP9: with fasting plasma glucose ($r=0.264$, $P<0.05$, $N=77$).
During the climacteric period in women the indexes of endothelium functioning (MMPs-9, TIMP-1, TNFα and CRP) decreased with the intensification of symptoms of metabolic syndrome: obesity, levels of fasting plasma glucose and levels of free testosterone index.

P267

New hepatic autoantigens in mice with APS-1 like disease

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The AIRE gene (autoimmune regulator) has been identified as an important mediator of central tolerance against ectopically expressed peripheral antigens. Mutatations of AIRE are responsible for autoimmune polyendocrinopathy syndrome type I (APSI) in human characterized by a multiorgan autoimmune disease. Autoimmune hepatitis (AIH) is part of the clinical spectrum in humans and mice. 19% of patients with AIRE mutations develop an autoimmune hepatitis which is characterized by autoantibodies against cytochrome P450 2A6 and 1A2. We investigated AIH in mouse models with various AIRE mutations on different genetic backgrounds. Initially we characterized the immunohistochemical staining pattern of sera from AIRE$-/-$ mice on rat liver, kidney and stomach sections and on HepG2 cells. In parallel, sera analysis has been done on western-blots loaded with whole liver lysate. We could demonstrate that the strength and broadness of the humoral immune response correlates well with disease activity of the AIH in AIRE deficient mice. We could show that AIH development is dependent on the genetic background used. Furthermore within the Balb/c background AIH development is dependent on the underlying AIRE mutation. Humoral immune responses in AIRE deficient mice are not directed against the antigens described for human AIH. We therefore set out to characterize the target antigens of the adaptive immune response in mice with AIH to study the defects in their immune tolerance. We therefore developed a column retention assay of for non-denatured liver antigens followed by a proteomics analysis with mass spectroscopy. With this approach we identified several potential new autoantigens of the humoral immune response.

P268

Thyroid gland hormonal disturbances after iodine excess among children

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Aim of the study was to evaluate influence of potassium iodide on hormonal thyroid function.
In medicine there are widely used medias with large, overphysiologic doses of iodine for both treatment and diagnosis. We investigated influence of high repeated iodine doses on hormonal thyroid balance in healthy children with no previous history of any thyroid diseases.
Thirty eight children at age from 6 to12 years old with no personal and familiar history of thyroid diseases, were treated with potassium iodide solution for a week as an oral pharynx disinfection. Indications were chronic pharynx infections. Evaluated patients had no contact with iodine during last twelve months. Thyroid hormone levels, antythyroid peroxidase antibodies and thyroid gland in ultrasound examination with 10 MHz sound were in normal range before larynx treatment. In all patients we measured levels of TSH and FT_4 before disinfection in the same day and after 4 and 12 weeks. Written informed consent was collected and study was approved by the Ethics committee. The collected results were submitted to a statistical study. Repeated measures ANOVA and posthoc analysis with LSD test were performed. $P<0.05$ was considered to indicate a statistically

significant difference. Statistical computations were executed with Statistica, commercially available Software (StatSoft USA).
The mean TSH and FT_4 levels of analyzed group stayed in laboratory normal range but changes were statistically significant. In ten out of thirty eight children we observed hypothyroidism after 1 month and they needed thyroxin treatment. Results of our study demonstrate that use of iodine at low risk thyroid disease children is not safe and makes serious complications. Potassium iodide in excess amounts should not be used among children. When applied, careful endocrinological and thyroid hormones control should be performed.

P269

The UV absorber 4-methylbenzylidene-camphor (4-MBC) causes effects comparable to primary hypothyroidism

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The endocrine active compound 4-methylbenzylidene-camphor (4-MBC) is frequently used as an UV absorber in sunscreens and various skin care products. Though 4-MBC is weakly estrogenic in the reproductive system of fishes and rodents it shows strong anti-osteoporotic effects. Furthermore, different studies show percutanous absorption of 4-MBC after dermal administration resulting in identical biotransformation in humans and rats. Female Sprague-Dawley rats received five concentrations of 4-MBC (10–600 mg/kg per b.w. days) via gavage over a period of five days on a background of a soy-free diet. As controls for the thyroid axis served T_4 and the antithyroidal drug methimazole (MMI), while E_2 was used to evaluate estrogenic effects. TSH, total T_4 and T_3 were measured by RIA. Transcript levels of genes involved in the feedback control in the pituitary (αGSu, Tshβ, 5′-deiodinases (Dio1, Dio2)) as well as gene expression of TSH receptor (Tshr), sodium-iodide symporter (Nis) and thyroid peroxidase (Tpo) in the thyroid gland were detected by Real-time RT-PCR. TSH serum levels were significantly elevated at concentrations $\geq$33 mg 4 MBC/kg while T_4 serum levels were slightly decreased, and T_3 levels remained almost unchanged, which is typical for the initial phase of hypothyroidism when the peripheral organs still maintain T_3 serum levels. In the pituitary αGSu and Tshβ were markedly increased at concentrations $>$33 mg/kg. Dio1 gene expression was down-regulated while Dio2 transcript levels were increased depending on the 4-MBC concentration applied. Moreover, the animals exhibited remarkably increased thyroid gland weights at concentrations exceeding 33 mg/kg. The data on increased gene expression of Tshr, Nis and Tpo in the thyroid gland were supported by immunohistochemistry. These data are consistent with decreased Dio1 activity in the liver, indicating that 4-MBC is a potent inhibitor of the pituitary–thyroid–axis.

P270

Serum level of vitamin A, transthyretine and retinol binding protein in a region polluted by polychlorinated biphenyls in a long term

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Vitamin A (retinol) plays a non-substitutable role in a process of growth and differentiation of tissues. Deficit of retinol can be encountered at its long-term diet deprivation (primary deficit), but it can occur also as a consequence of metabolism disorders on various levels (secondary deficit). Secondary deficit of retinol can occur also in people exposed to chemical substances in the long term or people living in an environment polluted by chemicals for a long time. One of the most significant environmental pollutants is polychlorinated biphenyls (PCB). Region of the Eastern Slovak Lowland belongs to regions with high exposure of inhabitants to PCB.
Material and methodology
Serum level of retinol, transthyretine (TTR) and retinol binding protein (RBP) was examined in 24 adults and 43 children from Strážske (PCB). Control group (KON) was comprised of 19 adults from the village Ždaňa and 40 children from Bardejov.
Results
In adults there was no significant difference in serum levels of retinol found. Serum level of TTR was within a normal range. Statistically significant differences were discovered in serum levels of TTR in children, in adults this difference was non-significant. In serum concentrations of RBP in children there were no statistically significant differences found, in adults this difference was significant. We assessed also the ratio RBP/TTR and estimated reserve of retinol in liver. In both cases we discovered significant differences in children as well as in adults.
Conclusion
PCB belong among the most significant disruptors of the environment. RBP together with the ratio RBP/TTR can be suggested as a biomarker of chronic pollution of the environment by PCB.

Endocrine tumours

P271

The comparison of serum VEGF levels between patients with metastatic and non-metastatic thyroid cancer, and patients with non-toxic multinodular goiter

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One of the important proangiogenic factors involved in the growth of normal and neoplastic tissues is vascular endothelial growth factor VEGF.
Therefore we hypothesized, that serum VEGF concentration would differ between patients with metastatic and non-metastatic thyroid cancer, with multinodular goiter and healthy subjects. We also hypothesized that endogenous TSH stimulation would effect serum VEGF level. The study protocol was approved by the Ethical Committee of the Collegium Medicum in Bydgoszcz, Nicolaus Copernicus University, Poland. All patients gave informed consent.
The study group consisted of 71 patients (62 females, 9 men), aged 44. 9 ± 12.3 year, with differentiated thyroid cancer, (50 papillary, 17 follicular and 4 oxyphilic), treated in our department in the years 2003–2006. All patients had undergone total or near total thyroidectomy and radioactive iodine treatment, that had resulted in remission in 59 patients and persistent/recurrent disease in 12 patients. The study included two control groups – 30 patients with non-toxic multinodular goiter and 30 healthy subjects.
Serum VEGF concentration was significantly higher in patients with distant metastases than in patients with remission or healthy. (423.4 vs 217.6 vs 235.55 pg/ml, $P<0.05$). This was not observed in patients with locoregional metastases. During endogenous TSH stimulation, VEGF significantly decreased (215.3 vs 169.6 pg/ml, $P<0.05$). Patients with multinodular goiter showed significantly lower VEGF concentrations than the remaining study groups.
Serum VEGF concentration might be used as an additional marker of thyroid cancer with distant metasases, but it's interpretation should be undertaken very cautiously. Endogenous TSH stimulation decreases VEGF levels in patients either with and without thyroid tissue, suggesting its regulatory effects through receptors located outside the thyrocytes.

P272

Special multiple endocrine neoplasia (MEN)

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The '*Multiple Endocrine Neoplasia*' predominantly affect the pituitary gland, parathyroids, thyroids, adrenal glands and pancreas. We present the case of a patient with three different functioning endocrine gland tumours, but who cannot be included in any of these groups.
Case report
Sixty-five-years-old female with recurrent episodes of nephritic colic and hypertension for 2 years; non-specific gastrointestinal complaints, hirsutism, hypertrichosis on the limbs, frontoparietal alopecia and increased libido.
Examination
BMI: 32 kg/m^2, androgenetic alopecia, facial hirsutism, hypertrichosis on the limbs, increased pilification midline; increased size of thyroid gland with nodule

on midline, 2 cm in diameter and of an elastic consistency that moves on swallowing. Vitiligo on hands.
Complementary
Calcium 10.6 mg/dl, phosphorus: 2.21 mg/dl. Calciuria: 394 mg/24 h; RTP: 74.8%, PTH: 95.6 pg/ml. FSH: 15.7 IU/l, LH: 7.42 IU/l, testosterone: 2.54 ng/ml, FAI: 19.63. SESTAMIBI gammagraphy: pathological deposit in upper right parathyroid projection area. Thyroid ultrasound: 2.4×2×1.5 cm diameter nodule on isthmus. Transvaginal ultrasound and abdominal CT: normal.
Total thyroidectomy and a parathyroidectomy for parathyroid adenoma were therefore performed, because a suspected malignant thyroid nodule was found, which was diagnosed as papillary thyroid carcinoma.
Post-surgical examination, clinical manifestations of hyperandrogenism persisted with testosterone of 3.52 ng/dl, FSH: 21.9 IU/l, LH: 11.7 IU/l; It was decided bilateral oopherectomy: the anatomopathological diagnosis was a steroid cell tumour in the right ovary (NOS), and the left ovary: hyperthecosis and cystic follicles.
Following the oopherectomy the patient clearly improving in terms of hirsutism and presenting a regrowth of hair. Testosterone: 0.12 ng/ml, FSH: 33.35 IU/l, LH: 11.36 IU/l.
Conclusion
This patient presented with: primary hyperparathyroidism, papillary thyroid carcinoma and functioning ovarian tumour. Although she cannot be included within any of the MEN groups that have been defined to date, this is an atypical clinical profile of multiple endocrine neoplasia.

P273

The early results of the treatment of well differentiated thyroid cancer and its dependence on chosen factors

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The aim of the study was to estimate the influence of a thyroid remnants' volume, postsurgical concentration of thyroglobulin and radioiodine dose on the early treatment efficacy of well differentiated thyroid cancer.
Material and methods
We retrospectively analyzed 91 patients (76 females, 15 men) with well differentiated thyroid cancer.
Results
Histological classification revealed 68.1% (62/91) papillary thyroid cancers, 25.3% (23/91) follicular thyroid cancers, and 6.6% (6/91) oxyphilic thyroid cancers. Among the group, 74 (81.3%) patients reached the remission criteria and the remaining 17 patients (18.7%) showed biochemical and morphological evidence of metastatic disease. The remission was obtained in 100% of patients in stage I of the disease, 68.4% – in stage II, 78.6% – in stage III and 33.3% in stage IV. The total radioiodine dose used in patients with remission, did not differ from the dose used in patients without remission. We did not observe the influence of remnant's volume on treatment efficacy, however larger remants required higher dose of radioiodine to obtain the remission. Patients with remission had lower postsurgical thyroglobulin concentration, than patients without remission. (22.2 vs 103.3 ng/ml, $P=0.00025$). NPV of Tg level <5 ng/ml was 100% and PPV of Tg >15 ng/ml was 39.35%.
Conclusions
Early treatment results of well differentiated thyroid cancer depend on the clinical stage, and postoperative serum thyroglobulin level, measured after endogenous TSH stimulation. Early treatment results are not dependent on age, sex, histological type of thyroid cancer, the dose of radioiodine used in brackets of 60–150 mCi and additional diseases. Total thyroidectomy is equally efficient as near total.

P274

Variability in rensponse to octreotide in patients with insulinoma detected by 111In-octreotide scintigraphy (Octreoscan)

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Purpose
111In-octreotide scinti aphy may be useful in patients with insulinoma during pre-surgical localization of the tumor and octreotide is effective in inhibiting insulin secretion and reducing the hypoglycemic events. The aim of the study was to evaluate 111in-octreotide scintigraphy in localizing primary and metastatic insulinomas and predicting the response to octreotide administration.
Patients and methods
Diagnosis of insulinoma was made in 17 subjects (M/F 9/8; aged 41.8±15.3 years) based on hyperinsulinemic hypoglycemia (mean IRI/BG ratio 0.91±0.23) during a 72-h fasting test and confirmed by surgical procedure (mean tumor diameter 1.3±0.4 cm). One patient had a malignant insulinoma. All patients had Octreoscan. Blood glucose and serum insulin were measured fasting and at 20, 40, 60, 80, 100, 120, 140, 160, 180 minutes during intravenous infusion of octreotide at a rate of 100 μg/hour for three hours (Intravenous Octreotide Suppression Test); response was considered: 'good' when mean insulin secretion during the test was suppressed over 50% of basal value, 'fair' when it was suppressed between 20 and 49%, and 'absent' when suppression did not reach the 20% of basal value.
Results
Octreotide inhibited insulin secretion in 14 on 17 patients (overall results: mean basal insulin 36.8±31.2 μUI/ml, mean insulin during the test 13.5±7.6 μUI/ml – $P<0.05$). Fifteen on 17 (88%) pancreatic insulinomas and 1 liver metastasis were localized with Octreoscan, among these patients 12 (80%) were responders (3 good responses and 9 fair responses) and 3 (20%) were non responders. Malignant insulinoma had a good response. The rate of suppression ranged from 0% to 84.1% (mean rate 34.7±25.2%).
Conclusions
According to the study 80% of insulinomas detected by 111In-octreotide scintigraphy may respond to octreotide administration with a very variable rate of inhibition of insulin secretion.

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Nerve vagus schwannoma

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Introduction
Schwannomas are benign, encapsulated, solitary, slow-growing tumors which originate from nerve sheath cells in cranial, periferic, symphathetic nerve system. Aproximately, 25–45% of schwannomas are in head and neck region. N. Vagus Schwannoma are seen relatively, rarely. The patients frequently apply with a slow-growing, painless servical mass. Malign transformation is unusual.
Case report
The patient 37 years old applied to our clinic because of mass on his neck.In his history, it was applied lymphadenopathy excision when he was 11 years old and it was applied servical radiotherapy because his pathology result showed Hodgkin lymphoma. The thyroid function tests were normal. It was found a colloidal nodul 4 cm in the right thyroid lobe and a 2 cm lymphadenopathy lateral to the right lobe in the neck ultrasonography. The result of fine needle biopsy that was applied from right thyroid lobe was evaluated as follicular neoplasm. It couldnot diagnose with fine needle biopsy from the mass that was in the right lateral neck. It was performed bilateral total thyroidectomy to the patient and It was performed excisional biopsy from the mass because of the close relationship with nerve vagus in the mass of right neck. At histopathologic examination it was found schwannoma in the nerve vagus and thyroid follicular adenoma. It wasnot attempt again because the mass was asemptomatic and had a close relationship with nerve vagus. The patient who doesnot have any complaint is still observed.
Discussion
It was found frequently in the vestibular nerve (acustic neuroma). The most of patients are between 20–50 years old. ıt was more common on women. Nerve Vagus Schwannomas are relatively less common. İt can be used fine needle biopsy and magnetic resonance imaging. The treatment is excision of the mass by protecting the nerve vagus. Nerve continuity can protect by mycrosurgical technique which was described by Fujino *et al.* In patients that underwent incomplete resection or have surgical contraindication the alternative cure methods are radiotherapy and sterotactic radio-surgery. The risk of malign transformation is extremely rare. The patient with Von Recklinghausen disease develop a malignant schwannoma in 3–13% of case but never before 10–20 year latent period. So the patients should be observed.

P276

Use of multispine computed tomography in diagnostics of ectopic acth-syndrome

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Aim
To access the efficiency of mulitispine computed tomography (MSCT) in ectopic ACTH-syndrome.
Patients and methods
Five clinical cases (2 female and 3 male cases) with severe hypercortisolism were analyzed, age from 26 to 63 years old.
Results
In all of the 5 cases there were significant reasons to suppose ectopic ACTH-syndrome: rapid development of clinical features (in 4 of 5 cases), loss of lean mass compound with rounded and plethoric face patients, presence of cutaneus hyperpigmentation (in 4 of 5 cases), hypopotassemia (in 3 of 5 cases), loss of muscle strength in all of the cases, increase (2) and high (3) values of ACTH combined with absence of adrenal or pituitary adenoma. Mean values of cortisol in blood were 8 am: 1422±1200 nmol/l, 11 pm: 1198±1270 nmol/l, ACTH 8 am: 168.6±148 pg/ml, ACTH 11 pm=176±174 pg/ml, free cortisol excretion in 24 h urine collection 3177±2471 nmol/l. The results of the functional tests also evidenced to ectopic ACTH-syndrome: negative high dose dexametazone test in 4 of 5 cases, low ACTH response to desmopressine injection (in 4 of 5 cases also). All of the cases were suspicious of ectopic ACTH-syndrome, so the search for the source of glucocorticoid excess was conducted. In all of the cases MSCT enabled to visualize the presence of tumor in lungs tissue from 2.5 to 7 mm in size, while positron emission tomography with FDG-18 which one of the patients previously underwent did not find the tumor.
Histological verification was made after the operation of atypical lobar lung resection. It revealed carcinoid tumor in two cases, mixed and small cell cancer in 3 cases. Immunohistochemical analysis shown positive reaction for ACTH in 30%–80% of tumor cells. Developing adrenal insufficiency in all of the patients confirmed radical effect of surgical treatment.
Conclusions
In absence of persuading evidence for pituitary or adrenal genesis of hypercortisolism, patients with high levels of ACTH should undergo desmopressine test and MSCT. So, MSCT may be defined as one of the prior methods of ACTH-producing tumors topical diagnostics.

P277

Rottlerin inhibits migration of thyroid follicular carcinoma through destablization of the focal adhesion complex

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The ability of local invasion and distant metastasis indicates malignancy. Focal adhesion complex plays an important role during cell invasion. This complex consists of integrins, focal adhesion kinase (FAK), vinculin, talin, α-actinin and paxillin. Activation of FAK regulates the assembly of focal adhesion and stress fiber formation via GTPase Rho and Rac, which is responsible for the migratory behavior of cells. In this study, we demonstrated the inhibitory effect of rottlerin on cell migration of an invasive follicular thyroid carcinoma CGTH W-2 cells via focal adhesion disassembly. Rottlerin treatment resulted in a two-fold decrease in the migratory activity of CGTH W-2 cells as estimated by both Transwell assay and wound healing assay. The protein expression levels of integrinβ1, active FAK, and active paxillin were decreased after rottlerin treatment. Consistent with these biochemical findings, disassembly of focal adhesions revealed by immunostaining for FAK, vinculin, and paxillin were noted. The disruption of actin stress fibers was seen, and was consistent with the reduced GTPase activities of Rac-1 and RhoA. This rottlerin-inhibited cell migration might not be attributed to the inhibition of PKCδ activity, since the inhibitory effect of rottlerin on cell migration could not be rescued by phorbol myristate acetate, which induced PKCδ activation and promoted the migration potential of CGTH W-2 cells. In summary, we demonstrated that rottlerin inhibits the migratory ability of CGTH W-2 by disassembly of focal adhesion complexes in a PKC δ-independent manner.

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The enzymatic activity of type 1 iodothyronine deiodinase (D1) is low in large intestine metastases into liver

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Type 1 iodothyronine deiodinase (D1) is responsible for the conversion of thyroxin (T_4) into tri-iodothyronine (T_3). The enzyme is mainly present in thyroid, liver and kidneys.
There is strong evidence that the metabolism of thyroid hormones is disturbed in some neoplasmatic tissues. However there are only few available data about D1 enzymatic activity in liver tumors.
The aim of this study was to estimate the enzymatic activity of D1 in metastases of large intestine carcinoma in comparison with healthy liver tissue. The activity was assessed by measurement of radioactive iodine released in reaction of deiodination catalyzed by D1.
Twenty-two tumors and twenty-two healthy control tissues, obtained from the patients (average age 61) operated because of second deposits in liver, were examined. It was found that D1 activity was significantly lower in metastatic tissues in comparison with healthy counterparts ($P<0.0001$).
This finding demonstrates low enzymatic activity of D1 in large intestine metastases into liver and suggest so far unknown role of thyroid hormones in this type of liver tumors.

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PTHrP and HRG outcomes in MCF7 breast cancer cells transfected with HER receptors gene

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Metastasis to bone occurs frequently in advanced breast cancer and is accompanied by debilitating skeletal complications. Parathyroid hormone-related protein (PTHrP) occurs in a high proportion of breast cancer and is strongly implicated in their metastatic spread to bone. Overexpression of PTHRP and its receptor in breast tumour cells could also promote the growth in an autocrine fashion. Signal transduction of growth factor receptor, EGFR (ErbB1, HER1) and ErbB2 (Neu, HER2) receptor has been implicated in conferring resistance to traditional chemotherapy on cancer cells. Activation of extracellular-regulated kinase/mitogen-activated protein kinase (Erk/MAPK) is a critical signal transduction event-mediated cell proliferation, cell migration and tumor progression. In the present study, we examined the role of PTHrP-1-34, PTHrP-7-34 and PTHrP-1-86 in the Erk pathway in MCF7 breast carcinoma cell line with or without heregulinbeta1 (HRG beta1). MCF7 were transfected with pcDNA3/EGFR or with pcDNA3/erbB2 and treated overnight with 10 nM of PTHrP-1-34, PTHrP-7-34, or PTHrP-1-86 and for 30 min with 10 ng of HRGbeta1 in serum free medium. PTHrP-1-34 significantly overexpressed ErbB1 and ErbB2 receptors compared with PTHrP-7-34 and PTHrP-1-86 treatment. Erk activity was significantly enhanced with HRG beta1 and PTHrP-1-34. But with PTHrP-7-34 and PTHrP-1-86, Erk activity was less significantly enhanced. Moreover, the level of protein kinase C, PKC found to be much higher with PTHrP-1-34 than PTHrP-7-34 and PTHrP-1-86 treated cells. However, the PKC was demolished in HRG beta1 treated cells and PKC activation had no effect on erbB1 and erbB2 induced Erk activation. Our results conclude that cross-talk between PTHrP receptor and ErbB1 and erbB2 receptors could be crucial growth-promoting effects in tumor progression.

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Type 1 iodothyronine deiodinase (D1) enzymatic activity is not reduced in liver focal nodular hyperplasia (FNH)

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FNH is the second most common benign liver tumor caused mainly by oral contraceptives. D1, which is a crucial enzyme in catalyzing prohormone thyroxine (T_4) into active triiodothyronine (T_3), is mainly present in such tissues as thyroid, liver and kidney. There are only few data about D1 enzymatic activity in neoplasmatic conditions. Mostly in tumors reduced D1 activity was found.
The aim of the study was to examine the activity of this enzyme in FNH.
Thirteen samples of the tumor and the corresponding number of healthy control tissues were collected from the patients, who underwent the surgery because of that disease. Average age was 28.3. We assessed the activity by measurement of radioactive iodine released in deiodination reaction catalyzed by D1.
We found that D1 enzymatic activity is not reduced in FNH in comparison with healthy controls ($P=0.555$). Our finding demonstrates that not every type of neoplasm presents low D1 activity.

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An unusual case of painful gynaecomastia due to large adrenocortical tumour

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We present an unusual case of painful gynaecomastia due to a large adrenocortical tumour secreting oestradiol and other steroid hormones.
A 46 years old man presented with a 6 months history of progressive, painful gynaecomastia. He had no other specific symptoms and had previously been well. Alcohol intake was not excessive. Interestingly, his sister had presented with a phaeochromocytoma 2 years previously.
Examination demonstrated bilateral gynaecomastia, numerous spider naevi, and palmar erythema. The liver and spleen were not palpable and no jaundice or ascites were evident. Beard, axillary and pubic hair was present. External genitalia were unremarkable. There were no features of Cushing's syndrome.
Serum concentrations of several steroid hormones were elevated: oestradiol at 887 pmol/l, testosterone 49.3 nmol/l, DHEAS 102.5 umol/l, and androstenedione >400 nmol/l. Urine free cortisol was 835 nmol/24 h, and serum cortisol at 0917 h was 580 nmol/l after overnight suppression with 1 mg of dexamethasone. Gonadotrophins were fully suppressed. Urine catecholamine excretion was normal. CXR showed a raised left hemidiaphragm, and CT scan confirmed the presence of a 17 cm left adrenal mass.
The left adrenal tumour was excised with perioperative corticosteroid cover. Serum hormone concentrations postoperatively included: oestradiol 95 pmol/l, testosterone 13.0 nmol/l, DHEAS 0.4 umol/l, LH 2.9 IU/l and FSH 3.1 IU/l. The patient's gynaecomastia and other features of abnormal oestrogenisation resolved.
This case presents a number of points which merit consideration. These include:
- A rare cause of rapid onset gynaecomastia.
- Rapid growth of the tumour to a large size, as reported previously.
- The possibility that the raised serum testosterone concentrations reported preoperatively might have resulted from an analytical interference in the presence of very high concentrations of DHEAS.
- The unusual association with a family history of phaeochromocytoma.

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Screening of MEN1 gene in patients with either classic or variant MEN1 presentation

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Multiple endocrine neoplasia type 1 (MEN1) is a rare dominantly inherited neoplastic syndrome. Tipically, it affects three major locations: parathyroid, endocrine pancreas or duodenum (GEP) and anterior pituitary. MEN1 is caused by mutations in MEN1 gene and its testing is now used as a complement to clinical diagnosis which may be hindered by the variable penetrance and expression of the defects. Mutation carriers are life-long monitored, while unaffected relatives can avoid expensive and distressing clinical screening. In this study, 51 patients with either classic MEN1 or MEN1-related diseases were screened for MEN1 germline mutations by sequences analysis. The patients were referred with the following clinical classification: 11 cases with classic MEN1 (hyperparathyroidism, pituitary adenoma and GEP tumor), 20 with variant-1 (2 out 3 classic lesions), 4 with variant-2 (multiple parathyroid tumors <30 years), 2 with variant-3 (pancreatic islet tumors), 14 with variant-4 (familial isolated hyperparathyroidism, FIHP). We found MEN1 germline mutations in 5/11 classic MEN1 cases (c.213delCAGA, p.L418R, p.R420X, p.F452S, c.1521delG), 4/20 variant-1 cases (p.R420X, p.R465X, c.317insGCCCC, p.F452S), 2/4 variant-2 cases (c.207delC, c.247_250delCTGT), 3/14 variant-4 cases (p.D158E in 2 cases, c.1937insC). The mutations p.D158E, c.1521delG and p.L418R are novel, while the other mutations were previously described in MEN1 cases. The novel deletion creates a frameshift in exon 8 and premature stop codon in a classic MEN1 case with multiple pancreatic lesions, including one gastrinoma, and a lung carcinoid. *In silico* analyses predict deleterious effects for L418R, but not for D158E. However, this latter variant was present in 2 unrelated cases with FIHP, while L418R was found in a classic MEN1 case. In conclusion, our results confirm the mutation frequencies in the different MEN1-related clinical settings, consistent with a fundamental role of DNA testing in the management of the patients affected with either classic or variant MEN1 presentation.

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Effect of treatment with depot somatostatin analogue octreotide on primary hyperparathyroidism in MEN-1 patients

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Background
Expression of somatostatin receptor (SST) and therapy with somatostatin analogues have been scarcely investigated in parathyroid tumors.
Objective
To evaluate the effects of depot long acting octreotide (OCT-LAR) on primary hyperparathyroidism in patients affected with multiple endocrine neoplasia type 1 (MEN-1).
Subjects and methods
Eight patients with a genetically confirmed MEN-1 were enrolled. All patients presented with primary hyperparathyroidism together with duodeno-pancreatic neuroendocrine tumors. A SST scintigraphy was performed in all patients before therapy with OCT-LAR. OCT-LAR 30 mg was administered every 4 weeks in all patients. Effects of OCT-LAR therapy on serum PTH, serum and urinary calcium and phosphorus were evaluated for 6 months.
Results
OCT-LAR normalized hypercalcemia and hypercalciuria in 75% and 62.5% of patients, respectively, while serum PTH levels were significantly decreased but still above the normal range. At SST scintigraphy, a positive parathyroid tumor uptake was found in 37.5% of MEN1 patients. The decrease of both serum PTH and serum and urinary calcium levels after therapy with OCT-LAR occurred regardless from the SST scintigraphy results.
Conclusions
OCT-LAR controlled hypercalcemia and hypercalciuria associated with hyperparathyroidism in two thirds of patients with MEN1-related parathyroid adenomas. However, these effects seem to be dissociated by the suppression of PTH levels.

P284

Correlation between postoperative hypothyroidism and seric homocysteine at the patients with thyroid carcinoma

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Carcinoma of the thyroid gland is an uncommon cancer but is the most common malignancy of the endocrine system. The treatment of the thyroid cancer includes surgery, radioactive iodine and hormone treatment. Postoperative hypothyroidism is the most common complication of thyroidectomy for thyroid cancer. After thyroidectomy, the patient takes a thyroid hormone drug (levothyroxine) in supresive dose for the rest of his life.
In hypothyroidism there has been reported an elevated plasma homocysteine. This may exacerbate the risk of cardiovascular disease in hypothyroidism that is traditionally attributed to lipid changes.

Combination therapy with L-thyroxine and multiple vitamins (B6, B12, folic acid) may correct plasma homocysteine in many patients.
Our study proposed following the changes after administration of folic acid daily. For this reason we chose a group of 20 patients with thyroidectomy for thyroid carcinoma, treated with ^{131}I, who take a thyroid hormone replacement drug.
Before the begining of the study, we measured the seric level of homocysteine, TSH, T_3, free T_4, cholesterol (total, LDL, HDL), trigliceride, Tg and AbTg. We also made carotidian doppler ultrasounds for measuring the intima – media thickness (IMT).
Every 3 months after the administration of the folic acid, there was made clinical, serological and imagistic evaluation of the patients.
After the first evaluations we observed the decrease of the seric level of homocysteine. IMT remained almost unchanged.

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The role of parathyroid hormone-related protein on gynecomatia in patients with Klinefelter's syndrome and idiopathic gynecomastia

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Background
Parathyroid hormone-related protein (PTHrP) was discovered as a tumor product. It is also one of the complex epithelial and mesenchymal interaction signal mediator in breast development. An increase in the prevalence of malignancy in patients with Klinefelter's syndrome, but not in patients with idiopathic gynecomastia has been previously reported. PTHrP may play a critical role in the development or progression of breast cancer.
Aim
We determined the PTHrP plasma level and investigated its potential role on idiopathic gynecomastia and gynecomastia which accompanies Klinefelter's syndrome.
Materials and methods
In this prospective clinical study, 40 patients with gynecomastia (15 with Klinefelter's syndrome, 25 with idiopathic gynecomastia) and 25 healthy controls were enrolled. Klinefelter's syndrome was defined with regard to clinical, biochemical findings and karyotyping. Idiopathic gynecomastia was defined as breast enlargement, which could not be related with any disease despite extensive investigations.
Results
PTHrP levels altered between the Klinefelter's syndrome and idiopathic gynecomastia groups (14.19 ± 3.29 in patients with Klinefelter's syndrome and 11.68 ± 2.10 in patients with idiopathic gynecomastia, $P = 0.031$). Serum alkaline phosphatase (ALP) level and sedimentation rate were higher in patients with Klinefelter's syndrome compared to patients with idiopathic gynecomastia (129 ± 32 U/l versus 97 ± 34 U/l, $P = 0.007$ for ALP and 8 ± 9 mm/h versus 2 ± 1 mm/h, $P = 0.001$ for ESR respectively).
Conclusion
Our findings established that PTHrP levels do not have any direct effect on gynecomastia development. There is a statistically significant difference between the PTHrP level of patients with Klinefelter's syndrome and idiopathic gynecomastia. The alterations in erythrocyte sedimentation rate and PTHrP may be an indicator of malignancy.

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Two new mutations in the RET protooncogene: R770Q in coincidence with Y791N in the same family with medullary thyroid carcinoma

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Context
Clinical studies are needed to classify rare and novel RET mutations associated with hereditary medullary thyroid carcinoma (MTC) into one of three clinical risk groups.
Objective
We analyzed genotype–phenotype correlations associated with the RET protooncogene mutation R770Q in exon 13 which was detected simultaneously with a Y791N mutation in the same family.
Results
Calcitonin determination in a 43-year-old female patient with multinodular goiter showed elevated levels (757 pg/ml; normal range < 11 pg/ml). CEA was also elevated (27.6 ng/ml (< 2.5 ng/ml). Ultrasound revealed a 2.2 cm hypoechogenic nodule on the left side. Plasma and urine catecholamines and metanephrines were in the normal range. RET analysis revealed a new mutation in exon 13 R770Q (CGA$>$CAA) in the patient. Screening of the sister of the index patient revealed surprisingly another, previosly not described amino-acid substitution Y791N (TAT791AAT) in the RET protooncogene. In the parents the R770Q mutation was detected in the mother, the Y791N mutation in the father. In the index case a thyroidectomy with central and lateral node dissection was done. Histology revealed MTC in a mixed variance with follicular cancer of 2 cm diameter, no lymph node involvement in 26 removed lymph nodes (T1N0M0). Postoperatively there is no increase of calcitonin after pentagastrin stimulation, the patient is biochemically cured concerning MTC. In all other gene carriers (aged 44–70 years), calcitonin levels were in the normal range, therefore, thyroidectomy had not yet been performed.
Conclusions
Our clinical findings indicate that the RET R770Q mutation may be associated with late-onset nonaggressive disease. For the Y791N mutation no association with MTC could be detected, although other exchanges at position 791 are known causes for MTC. Recommendations for prophylactic thyroidectomy should be individualized depending on stimulated calcitonin levels.

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The role of 2-[^{18}F]-fluoro-2-deoxy-D-glucose positron emission tomography (FDG/PET-CT) in the follow-up of differentiated thyroid cancer (DTC)

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Aim
To address the role of FDG/PET-CT in the follow-up of DTC.
Methods
About 110 consecutive patients (86 female, 24 male, mean age 45 ± 13 years), with DTC were selected between 1999 and 2006. All patients underwent total thyroidectomy, radioiodine ablation and had undetectable serum thyroglobulin (Tg) during L-T_4 suppressive therapy and negative serum TgAb. The follow-up included a yearly clinical examination with neck ultrasonography (US) and Tg determination after rhTSH stimulation. All subjects with elevated Tg and negative US underwent FDG/PET-CT.
Results
Tg levels were undetectable during TSH-suppressive therapy in all patients. After rhTSH, Tg values remained undetectable in 81 patients, whereas 29/110 became detectable during follow-up.
At 12–24months, 8/29 cases showed low detectable Tg values (< 2 ng/ml).US detected suspicious cervical lymph node metastases in three cases confirmed by FNAC, while Tg values became undetectable during subsequent surveillance in the other 5 cases.
At 24–36 months, 7/29 patients showed serum Tg $> 2 < 5$ ng/ml and underwent US and FDG/PET-CT. Suspicious cervical lymph node metastases were detected in 2 patients by US and 1 by FDG-PET, confirmed by FNAC, and 4 patients with negative US and PET went through a Tg stimulation test after TSH withdrawal 6–12 months later. Four patients showed a persistent Tg increase but only one tested positive in the lung by PET/TC.
At 36–60 months, 14/29 cases had serum Tg levels > 5 ng/ml: 4 showed uptake in the mediastinal region, 4 in the cervical region, 1 case in the lung and 1 both in the cervical and mediastinal regions by FDG/PET-TC. The remaining 4 patients tested negative through additional imaging (CT, MRI).
Conclusion
During the first years of follow-up, US was highly sensitive in detecting recurrence of cervical disease. FDG-PET-CT scan is a useful diagnostic test to detect and localize metastases when post-rhTSH Tg is > 5 ng/ml in the later period of follow-up.

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Effects of the potent deacetylase inhibitor Panobinostat (LBH589) against poorly differentiated and anaplastic thyroid cancer cells

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Poorly differentiated and anaplastic thyroid carcinomas are aggressive human cancers that are resistant to conventional therapy. DAC inhibitors (DACi) are a promising class of drugs, acting as anti-proliferative agents by inducing apoptosis and cell cycle arrest.
Panobinostat (LBH589) is a potent DACi belonging to a structurally novel cinnamic hydroxamic acid class of compounds, able to inhibit the activity of different isoforms of HDACs (HDAC 1, 3–6) and the proliferation of tumour cells at nanomolar concentration.
Aim of the present work was to evaluate the effect of panobinostat on the growth of poorly differentiated and anaplastic thyroid cancer cell lines. Cells were treated with increasing doses of panobinostat (0–5–10–25–50–100–200–500 nM) up to 72 h. After treatment, we evaluated: (1) cell viability and cytotoxicity by WST-1 method; (2) cell cycle progression by FACS analysis. Our data demonstrate that panobinostat has cytotoxic effects on both poorly differentiated and anaplastic thyroid cancer cell lines, and this is through G_2 cell cycle arrest and apoptosis induction. Moreover, panobinostat is able to induce acetylation of histones and tubulin in both types of cell lines, as demonstrated by western blot analysis. These data suggest a mechanistic link between histone and tubulin acetylation, and the panobinostat-induced cytotoxic effects. This study supports the rationale for further clinical development of panobinostat as a potential therapy for the treatment of poorly differentiated and anaplastic thyroid cancers.

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Cytoplasmic shift of AUF1 in thyroid carcinoma

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AUF1/heterogeneous nuclear ribonucleoprotein D (hnRNPD) is an adenylate uridylate-rich elements (ARE) binding protein, which regulates the mRNA stability of many genes related to growth regulation, such as proto-oncogenes, growth factors, cytokines and cell cycle regulatory genes. Several studies demonstrated AUF1 expression in kidneys, liver, lymphoid tissues and melanocytes, and its involvement in apoptosis, tumorigenesis and development by its interactions with AREs bearing mRNAs. In this study we provide evidence, that AUF1 may be related to thyroid carcinoma progression. We could show that AUF1 can influence the cell cycle of thyroid carcinoma cell lines and its cytoplasmic and nuclear expression varies during cell division. Moreover knock-down of AUF1 led to increased levels of retinoblastoma protein and down-regulation of wild type p53, and elevated expression of cyclin-dependent kinase inhibitors, what correlated with decreased proliferation rate of thyroid carcinoma cells. By subcellular fractionation of thyroid tissues and immunohistochemistry we could show that cytoplasmic expression of AUF1 in benign and malignant tissues was significantly increased when compared to normal thyroid tissues. Moreover logarithmic nuclear/cytoplasmic ratio of total AUF1 expression in normal, goiter, adenoma and follicular thyroid carcinoma decreased with tissue malignancy.
Our data suggest that AUF1 may be a regulator and/or mediator of the expression of many mRNAs, involved in thyroid carcinoma progression supporting hypothesis that its increased cytoplasmic expression may promote thyroid carcinogenesis. This is the first report demonstrating the expression of AUF1 in normal, benign and malignant thyroid tissues indicating that AUF1 could serve as a novel marker for thyroid carcinoma.

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Down-regulation of ENO1/MBP-1 gene products by retinoic acid (RA) causes decreased proliferation of the follicular thyroid carcinoma cell line FTC-133

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Retinoic acid (RA) acts as an anti-proliferative and re-differentiation agent in the therapy of thyroid carcinoma but the molecular mechanisms by which RA mediates these effects are not well understood. We have investigated the effect of RA on the production and post-translational modification of the two ENO1 transcriptional products in the human follicular thyroid carcinoma cell line FTC-133. The single ENO1 transcript encodes a 48 kDa ENO1 with its unique N-terminal enolase activity and a truncated 37 kDa MBP-1 which, like ENO1, contains a C-terminal c-*myc* promotor-binding domain. RA treatment of FTC-133 caused a down-regulation of both ENO1 gene products. Two-dimensional gel detection and mass spectrometric analysis revealed that ENO1 existed as three separate protein spots of distinct isoelectric points (ENO1-A1-A3). Comparative 2D gel analysis of fluorescently labelled protein samples of RA treated and untreated FTC-133 (DIGE) demonstrated a selective down-regulation of ENO1-A1 which we identified as a phosphoprotein. RA caused the dephosphorylation of ENO1-A1. The down-regulation of ENO1 and MBP-1 correlated with reduced intracellular ATP levels and the down-regulation of c-Myc oncoprotein. Specific knock-down of ENO1 resulted in a marked reduction in proliferation of FTC-133. Thus, the RA-induced down-regulation and post-transcriptional modification of the ENO1/MBP-1 gene products may mediate its anti-proliferative effect by distinct molecular mechanisms, including a decrease in enolase activity and suppression of c-Myc oncogene. The glycolytic enzyme and novel RA target molecule ENO1 can facilitate both these mechanisms and may be considered a new marker in human thyroid carcinoma.

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Differences in the gene expression patterns of several immune related genes in various human adrenocortical tumors

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Introduction
The adrenal cortex is highly involved at the immune-neuroendocrine interface, and several cytokines are known to influence the proliferation, differentiation, apoptosis and hormone production of adrenocortical cells. We therefore hypothesized that immune mediators might be involved in adrenal tumorigenesis, as well. Gene expression patterns of various inflammatory and immune mediators were studied by a functional genomics/bioinformatics approach.
Methods
Hormonally inactive, cortisol-, aldosterone-secreting benign and malignant adrenocortical tumors, and normal adrenocortical tissues were studied by microarray analysis. Both 8×1.5 K custom microarrays focusing on immune-related genes and whole genome 4×44 K microarrays were performed in 6–10 samples in each group. Real-time RT-PCR and western-blotting were used for the validation of results.
Results
Seventy genes showed significant ($P<0.05$, min. 2–2.5 fold) differences among different groups by microarray analysis. Among these, toll like receptor 4 (TLR4), macrophage migration inhibitory factor (MIF), fibroblast growth factor 11 (FGF11), fibroblast growth factor receptor 1 (FGFR1), interleukin 17 (IL-17) and IL-17 receptor beta (IL-17RB) expression levels were validated. TLR4 expression was highest in normal tissues and hormonally inactive tumors, while low

expression levels were measured in the other groups. MIF and FGF11 expression levels were characteristically high in hormonally inactive tumors, whereas barely detectable in the other groups. In contrast, FGFR1 was highest in normal tissues, low expression was found in tumors. IL-17 expression was not significantly different, whereas IL-17RB expression was significantly reduced in inactive tumors.
Discussion
Our findings underline the possibility that some immune related genes may be involved in the pathogenesis of adrenocortical tumors.

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Different gene expression patterns of histamine related genes in human adrenocortical tumors

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Introduction
The pathogenesis of sporadic adrenocortical tumors is poorly elucidated. Considering the importance of the adrenal cortex at the immune-neuroendocrine interface and the known actions of immune mediators on adrenocortical cell functioning, we supposed that immune or inflammatory factors might be involved in the pathogenesis of these tumors. Gene expression patterns of various inflammatory and immune mediators were studied by a functional genomics/bioinformatics approach.
Methods
Hormonally inactive, cortisol-, aldosterone-secreting benign and malignant adrenocortical tumors, and normal adrenocortical tissues were studied. Both 8×1.5 K custom microarrays focusing on immune-related genes and whole genome 4×44 K microarrays were performed in 6–10 samples of each group. Real-time RT-PCR and western-blotting were used for the validation of results.
Results
Seventy genes showed significant ($P<0.05$, min. 2–2.5 fold) differences. Among these, histidine decarboxylase (HDC) and histamine receptors were noteworthy, as no previous data described the involvement of histamine in adrenocortical tumorigenesis. Therefore, we examined and validated the expression of all genes involved in histamine biosynthesis, action and degradation. HDC expression was highest in normal tissues, whereas hardly detectable in tumor samples. Histamine H2 receptor expression was the highest in normal tissues too, its expression was lower in hormonally inactive, and much lower in hormone-secreting and malignant tumors. Histamine H4 receptor could not be detected in cortisol-secreting tumors, however, it was moderately expressed in other samples.
Discussion
Our findings raise the possibility that adrenocortical tumorigenesis might be characterized by reduced histamine biosynthesis and action.

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The prevalence of chosen complications in 72 acromegalics

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Introduction
Hipersecretion of somatotrophin axis hormones in acromegaly favours the development of numerous metabolic and organ complications. The aim of the study was to establish the occurrence integrity of these complications in acromegalics.
Material and methods
The tested 72 acromegalics – 32 men aged 46.9 ± 15.2 years and 40 women aged 58.8 ± 12.0 years, for which the period of the illness was determined, the body mass index (BMI) and the waste-hip ratio (WHR) were calculated, and the serum concentration was determined for: the growth hormone (GH), insulin-like growth factor 1 (IGF-1) and other hormones. Ultrasonography tests of thyroid, heart, abdominal cavity and pelvis were conducted. All the patients were proposed colonoscopy test, however only 51 of them gave their consent to it.
Results
A frequent occurrence of abnormal body weight (overweight and obesity) in 64 (89%) acromegalics; cardiovascular complications in 56 (78%) including hypertension in 35 (49%), cardiac muscle hypertrophy in 34 (47%) and myocardial ischemia in 13 (18%); disturbances of glucose metabolism in 50 (69%) including impaired glucose tolerance in 20 (28%), diabetes in 17 (24%) and abnormal fasting glucose in 12 (17%); abnormalities in lipid metabolism in 50 (68%), nodular goitre in 37 (51%), parenchymatous goitre in 12 (17%), benign prostate hypertrophy in 16 (50%) of the men, uterine myomas in 19 (48%) of the women, cysts of organs in 12 (17%) including renal cysts 7 (10%) and cholelithiasis in 20 (28%) of patients was registered. During the colonoscopy polyps were shown in 21 (41%) including adenomas in 10 (20%), hyperplastic polyps in 9 (17.3%) and inflammatory polyps in 4 (7.7%) of testated acromegalics.
Conclusions
Our study revealed high percentage of metabolic and organ complications, that may increase risk of mortality in the future in these patients.

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Expression of the somatostatin receptors (SSTRs) in patients with gastro-pancreatic neuroendocrine tumours (GEP-NET) and medullary thyroid cancers (MTC)

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The pattern of SSTRs expression in neuroendocrine neoplasms determines the possibility of the detection of the primary tumour and distant metastases, the application of radio-guided surgery (RGS) and the outcome of the treatment with itrium or lutetium labeled somatostatin analogues. The frequency and expression pattern of each subtype of SSTRs not only vary considerably in different NET but also in each patient.
The aim of the study was to assess the SSTR expression pattern in GEP-NET and MTC patients.
Material and methods
The study included 32 patients diagnosed with GEP-NET (14 with gastrointestinal, 8 with pancreatic and 2 with bronchial NETs) or MTC (8 patients). (99mTc-EDDA/HYNIC)octreotate (somatostatin analogue with high affinity to SSTR2) scintigraphy (SRS) was performed in all patients before surgery. SSTR1-SSTR5 expression was assessed immunohistochemically in paraffin embedded tumour tissues with use of polyclonal antibodies (Gramsch-Schwabhausen, Germany). Expression intensity was assessed in 0–3 semi-quantitative scale.
Results
The SSTR1 was detected in 56.3%, SSTR2 in 87.5%, SSTR3 in 31.3% and SSTR5 in 53.1% of samples. No tumour showed the expression of SSTR4. No SSTRs expression was found in five patients (four GEP-NETs, one MTC).
Preoperative SRS was negative in 6 patients, which correlated with no or very weak expression of SSTRs. The target/non-target ratio (SRS) did not correspond with SSTRs expression intensity. Surprisingly, in some patients with relatively low expression of SSTR2 high quality SRS was obtained. In 5 patient with positive SRS, RGS was performed, which enabled localization of preoperatively occult primary or metastatic lesions – all of them showed expression of SSTR2 in tumour tissues. Eight patient of the study group underwent palliative 90Y-DOTATATE therapy.
Conclusions
No SSTRs expression results in false-negative SRS. Even weak positivity for SSTRs may enable detection of the occult primary and metastatic NET lesions with RGS and further treatment with 'hot' somatostatin analogues.

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Melatonin restores the basal level of lipid peroxidation in rat tissues under conditions of exposure to potassium bromate

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Potassium bromate ($KBrO_3$) is a known prooxidant and carcinogen. Melatonin is a highly effective antioxidant. Indole-3-propionic acid (IPA) – an indole substance, and propylothiouracil (PTU) – an antithyroid drug, also reveal some antioxidative effects. The aim of the study was to evaluate $KBrO_3$-induced lipid peroxidation *in vitro* in tissues collected from either control or melatonin-treated rats, and to compare potential preventive effects of melatonin, IPA and PTU.
Kidney, liver and lung homogenates from either the control or the melatonin-treated rats (0.0645 mmol/kg b.w., i.p., twice daily, for 10 days) were incubated in the presence of $KBrO_3$ (0.1, 0.5, 1.0, 2.5, 5.0, 10.0 mM). As prooxidative effect of $KBrO_3$ was observed only in the control lung homogenates, they were then incubated in the presence of $KBrO_3$ (10.0 mM) plus melatonin (0.01, 0.1, 0.5, 1.0, 5.0, 7.5 mM), or IPA or PTU (0.01, 0.1, 0.5, 1.0, 5.0, 7.5, 10.0 mM). The level of lipid peroxidation products – malondialdehyde +4-hydroxyalkenals (MDA+4-HDA) – was measured spectrophotometrically.
$KBrO_3$ treatment increased lipid peroxidation in the control lung homogenates, but not in the lung from the melatonin-treated rats. Melatonin, IPA and PTU reduced $KBrO_3$-induced lipid peroxidation. Unexpectedly, $KBrO_3$ caused a concentration-dependent decrease in lipid peroxidation in liver and kidney homogenates from the control rats, whereas such an effect was less pronounced in tissues from the melatonin-treated rats.
In conclusion, $KBrO_3$-induced lipid peroxidation in rat lung suggests that it may be the target organ for this carcinogen. An exposure of an organism to melatonin decreases tissue sensitivity to $KBrO_3$-induced damage, possibly by restoring the oxidative balance. Thus, melatonin could be recommended for its possible cancer prevention effects.

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Relaxin and S100A4 alter the invasive potential in estrogen-independent human MDA-MB-231 breast cancer cells

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The heterodimeric peptide hormone relaxin is involved in extracellular matrix turnover during development and lactational differentiation of the breast. Relaxin expression is increased in human breast cancer and serum relaxin levels in breast cancer patients were reported to correlate with metastatic disease. We have established relaxin over-expressing transfectants of the highly invasive estrogen receptor-negative human breast cancer cell line MDA-MB-231 (MDA/RLN) to investigate the role of relaxin in estrogen-independent breast cancer cells. MDA/RLN revealed decreased cellular motility and migration through extracellular matrices when compared to MDA/EGFP control transfectants. S100A4 (metastasin) increased cell motility in different cell models and has been associated with tumor invasiveness. In the present study, we showed that the calcium binding protein S100A4 is down-regulated in stable MDA/RLN transfectants and in paternal MDA-MB-231 breast cancer cells after exposure to human recombinant relaxin. Furthermore, tumor growth in nude mice was studied following subcutaneous injection of MDA/RLN and MDA/EGFP clones and revealed reduced tumor growth of the MDA/RLN xenografts. MDA/RLN xenografts also revealed a down-regulation of S100A4 transcripts and protein. S100A4 siRNA knock-down revealed reduced cell motility suggesting S100A4 to be a downstream target of relaxin/ relaxin receptor signaling. (Supported by Wilhelm-Roux-Program, FKZ 15/09)

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The Relaxin peptide is a novel regulator of S100A4 (metastasin) in thyroid carcinoma cells

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The peptide growth factor relaxin (RLN) is expressed in human thyroid carcinoma cell lines and tissues. We have previously established thyroid carcinoma cell transfectants with over-expression of relaxin and demonstrated that relaxin increases cellular motility and in-vitro invasiveness in these transfectants. This increase in motility and migration is relaxin receptor (LGR7)-dependent. S100A4 (metastasin), a member of the S100 family of calcium binding proteins, is known to confer invasiveness in breast cancer cells and its expression in carcinoma tissues correlated with advanced stages of the disease. S100A4 is considered a molecular marker for the metastatic potential for carcinoma with high prognostic significance. We have shown that S100A4 is increased in thyroid adenoma, in papillary, follicular and anaplastic thyroid carcinoma tissues as compared to goiter tissues and S100A4 protein is localized in the cytoplasm of tumor cells. Highest intra-tumor levels of S100A4 expression were detected in papillary thyroid carcinoma with nodal metastasis. We show here that the relaxin/LGR7 ligand-receptor system is a novel transcriptional regulator of S100A4 expression in thyroid carcinoma cell line FTC133. The relaxin-mediated increase in motility is mediated by an increased expression of S100A4 mRNA and protein. Furthermore, S100A4 siRNA knock-down prevented the relaxin-induced increase in cellular motility of the cell line FTC-133. Thus, relaxin expression is a novel regulator of S100A4 in thyroid carcinoma cells and S100A4 may mediate the relaxin-induced increase in cancer cell invasiveness.

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Cyclic Cushing disease: clinical case

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Cyclic Cushing's disease is a rare situation due to episodic hypersecretion of ACTH. Suspicion is raised when strong clinical stigmata occur, with normal basal values of cortisol and normal responses to dynamic tests. After performing several tests, particularly during phases without symptoms (well-being), the probability of successful diagnosis increases.
We describe the case of a 33 years old female patient with full-blown clinical picture (weight excess, hirsutism, hypertension, muscular weakness, moon facies, buffalo hump and supraclavicular fat pads) since about a year. Repeated FUC showed fluctuating values – 112, 200 and 68 μg/24 h (*N*: 10–80). Plasma cortisol values at 8 am and 11 pm were respectively 11 μg/dl and 13 μg/dl. ACTH was 26 pg/ml at 8 am and 34 pg/ml at 11 pm. Low dose DXM suppression test suppressed plasma cortisol to 1.1 μg/dl. Pituitary MRI suggested a 'pars intermedia cyst'. An Inferior petrosal sinus sampling study (IPSS) was performed in an asymptomatic phase: ACTH reached 703 pg/ml in the right side and 541 pg/ml in the left side. Patient was submitted to transsphenoidal surgery (pathology: corticotrophinoma). After surgery, blood pressure normalized, hirsutism and body weight decreased. Six weeks after surgery, hormonal study showed: ACTH 10 pg/ml (8 am) and 7.4 pg/ml (11 pm); cortisol 7.4 μg/dl (8 am) and <1 μg/dl (11 pm); Cortisol after overnight 1 mg dexamethasone suppression test <1.0 μg/d;. There were no other pituitary hormonal disturbances. Three months later, MRI was repeated, with no evidence of relapse or tumoral residue.
Conclusions
The strong suspicion of Cushing syndrome justified keeping on with imagiologic and hormonal studies, in spite of normal DXM suppression test and slightly elevated FUC at the beginning. The hypercortisolism alternating with normal hormonal secretion suggests that we are facing a cyclic Cushing syndrome.

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Adrenal ganglioneuroma: a new cause of increased serum calcitonin

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Introduction
Ganglioneuromas are rare, benign tumors of mature ganglion cells, arising from the sympathetic ganglia and 20% are located in the adrenal medulla, representing 1–4% of adrenal incidentalomas. About 1/3 secrete catecholamines but hypertension and other adrenergic symptoms are rare. About 60% are depicted with ^{131}I- MIBG.
Case report
A 72-year-old man was admitted for a left adrenal tumor 9×8 cm, incidentally found in abdominal CT scan for investigation of haematuria. The patient was normotensive and diabetic type II. Basic and dynamic tests for cortisol and aldosterone hypersecretion were normal, 24 h urinary normetanephrine (2070 μg (105–354)), norepinephrine (422 μg (20–100)) and dopamine (425 μg (80–350)) were elevated (normetanephrine was 7 times the upper normal limit). Urinary epinephrine and metanephrine were normal. ^{131}I- MIBG whole body scan showed an intense uptake by the mass. Serum CgA was increased (334 ng/ml (<98)). Serum calcitonin (121 pg/ml (<15)) was increased without ultrasound apparent thyroid nodules. The tumor was diagnosed as a pheochromocytoma and a left adrenalectomy was performed with no perioperative complications. Histology revealed a gaglioneuroma with mature ganglion cells and abundant Schwann cells. Immunohistochemical staining for calcitonin was positive. Six months postoperatively, urinary catecholamines and normetanephrines were normal, abdominal CT scan showed a normal right adrenal, ^{131}I- MIBG scan was negative and serum CgA decreased to almost normal levels (102 ng/ml). Calcitonin also decreased to normal (4.6 pg/ml).
Conclusion
Untill now only pheochromocytomas and composite pheochromocytomas – tumors containig both chromafine and ganglion cells – are reported to secrete calcitonin. To our knowledge this is the first reported ganglioneuroma secreting calcitonin.

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Proteomic profile of GH-secreting versus non-functioning pituitary tumors

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GH-secreting and non-functioning pituitary tumors are clinically distinct, usually benign but potentially locally aggressive lesions originating from the replication of a single mutated pituitary cell. As for the underlying genetic and epigenetic alterations, also the patterns of activation of specific signaling pathways as well as the prognostic molecular factors leading to local invasiveness are, to date, largely unknown. In this study, we used two-dimensional electrophoresis and mass spectrometry to directly analyze protein profiles changes between GH-secreting (GH-omas) and non-functioning (NFPAs) pituitary tumors. In particular we analyzed 10 NFPA and 10 GH-omas surgically removed from patients not previously treated either by radiotherapy or somatostatin analogues. Proteins analysis has been performed by comparing ten gels obtained from each group of surgically eradicated tumors. Forty-two significant spots have been analyzed by mass spectrometry, resulting in the identification of eleven proteins significantly down-regulated and twenty-five proteins significantly up-regulated in non-functioning pituitary tumors in comparison to GH-secreting tumors. In particular, non-functioning pituitary tumors are characterized by a significant increase in Insulin-like growth factor-binding protein 2, Ubiquinol-cytochrome-*c* reductase complex core protein 1 and GTP-binding nuclear protein Ran expression. Interestingly, these proteins, whose function has not been yet investigated in human pituitary, are typically associated with a more aggressive tumor phenotype. These preliminary data to be further investigated, seem to suggest a more aggressive phenotype of non-functioning pituitary tumors compared with GH-secreting pituitary tumors. Experiments comparing the normal pituitary protein expression pattern with secreting and nonsecreting pituitary tumors are in progress.

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Genetic testing of *RET* protooncogene in multiple endocrine neoplasie type 2 and medullary thyroid carcinoma

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Introduction
Multiple endocrine neoplasia type 2 (MEN2) and medullary thyroid carcinoma (MTC) are autosomal-dominant inherited diseases caused by germline mutations within the *RET* protooncogene. Until now, genetic testing for mutations of exon 10, 11, 13, 14, 15 and 16 was recommended for these patients (familial and sporadic cases) to identify disease-causing mutations. Because of a strong genotype–phenotype correlation in these diseases, early genetic testing of relatives of the affected gene carrier is important to detect asymptomatic gene carriers to plan thyroid management e.g. total thyroidectomy.
According to 'Consensus Guidelines for Diagnosis and Therapy of MEN1 Type 1 and Type 2' we started to expand genetic testing for *RET* mutations within the remaining 15 exons of the *RET* protooncogene in 100 unrelated apparently sporadic index cases tested negative for exon 10, 11, 13, 14, 15 and 16. Thus, we want to get knowledge about the frequency and localisation of mutations within other regions of the gene to improve genetic testing strategy if necessary.
Methods
Genomic DNA was extracted from peripheral blood leukocytes, followed by PCR amplification and direct sequencing of exons including corresponding exon–intron boundaries.
Results
Until now additional analysis of six exons revealed two novel heterozygous germline mutations in exon 8: p.Ala513Gly and p.Arg552Gln. In addition, we detected a *RET* mutation p.Thr338Ile known from a study of Hirschsprung's disease.
Conclusion
So far, we detected three *RET* mutations in three out of 100 apparently sporadic patients tested negative for exon 10, 11, 13, 14, 15 and 16.
Our data indicate by now that genetic counseling and genetic testing for *RET* mutations should include rare mutations and be done in familial and apparently sporadic MTC/MEN2 index cases to confirm the diagnosis and define asymptomatic gene carriers for early therapy.

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Impact of the use of recombinant TSH stimulated thyroglobulin measurement on health related quality of life in patients in follow-up for differentiated thyroid carcinoma (DTC)

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Introduction
Thyroglobulin (Tg) measurement is the cornerstone in the follow-up of DTC. Sensitivity can be optimised by measuring recombinant TSH stimulated Tg (rhTSH-Tg). Higher sensitivity results in more Tg positive patients who need imaging and considerable patient burden. We assessed the impact of rhTSH-Tg measurement on health related quality of life (HRQOL).
Methods
In 121 patients in follow-up for DTC, Tg during thyroid hormone suppression therapy (Tg-on) and rhTSH-Tg were measured with a sensitive Tg assay. Patients with rhTSH -Tg≥1.0 ng/ml (Tg+ patients) underwent imaging. Anxiety items of the Hospital Anxiety and Depression scale, the General Health Questionnaire (psychological distress) and cancer worries (CW) were completed at baseline and after being informed about rhTSH-Tg result. Additionally, Tg+ patients completed these questionnaires after being informed about imaging results and disease status. Non-parametric statistic tests were used.
Results
RhTSH-Tg measurement resulted in 101 Tg− and 20 Tg+ patients. Imaging showed recurrence in 3/19 Tg+ patients. Two of these three patients could have been identified solely by Tg-on. In 16/19, Tg+ patients imaging was negative. For Tg+ patients, anxiety, psychological distress and CW significantly increased after being informed about Tg result. Only CW remained increased after being informed about disease status. For Tg− patients, anxiety decreased after being informed about rhTSH-Tg result, no other changes were found.

Conclusion
Notification of positive rhTSH-Tg has a negative effect on HRQOL and increment of CW persists despite of negative imaging. The limited clinical benefit and adverse effects on HRQOL of rhTSH-Tg measurement questions the use of this test in the follow-up of DTC. Tg-on measurement with a sensitive Tg assay may represent a useful diagnostic tool, preventing needless patient burden.

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Cushings' syndrome due to ectopic ACTH secretion: four cases

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Ectopic ACTH syndrome (EAS) occurs in around 10% of all cases with ACTH-dependent hypercortisolism. The mean age of clinical presentation varies from 45 to 50 years, and most of them caused by intrathoracic neoplasm, and recognition of the disorder may be delay. Subsequently, it may be difficult to locate the ACTH source and manage the patients' hypercortisolism. We present our patients with EAS from 2000 to 2007. Four patients, aged 15–43 years, two females and two males included in the study. Symptoms duration lasted from 5 months to 4 years. All patients presented clinical signs of hypercortisolism with intense weakness due to proximal myopathy, 3 presented arterial hypertension, 1 presented poor controlled diabetes mellitus for 8 months, none of them presented hyperpigmentation, and two patients presented a mild psycotic picture. Hypokalemia was present in 2 cases (<3 mEq/l), all of them presented absence of cortisol circadian rhythm. Considering the upper limit of normal values for the assay, plasma ACTH levels were within normal range in 3 and slightly high in one. Three had osteoporosis and fracture. None of them did respond to 8-mg 2-d high-dose dexamethasone suppression test. Chest X-ray and CT localized the ACTH-secreting tumor in only one patient, and diagnosis proved by biyopsy. All imaging modalities (CT/MRI/octreotide scintigraphy) were negative in the others. Two patients did not respond to CRH, and whereas one of these patients and another one did not showed a central-to-peripheral ACTH gradient on IPSS. Tumor markers, such as calcitonin, gastrin, CEA, and 5-HIAA were also negative in all patients. One patient had curative surgery, and three patients underwent bilateral adrenalectomy for control hypercortisolism. Subsequent imaging with CT/MRI.
Octreotide scintigraphy finally pointed to the presence of these tumors (2 pulmonary ACTH-secreting tumor, and one pancreatic neuroendocrine tumor) from 4 months up to 7 year later. One out of three are alive. Although initial failed localization is common, survival is good among the patients with pulmonary ACTH-secreting tumors.

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Inhibition of intracellular signaling pathways and induction of cell cycle arrest by the multi-kinase inhibitor sorafenib in thyroid carcinoma cells

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Objective
Therapeutic options for patients with dedifferentiated thyroid carcinoma are rare and alternative treatment strategies are needed. Among the most promising new agents for these patients are protein kinase inhibitors like the BRAF- and multi-targeted kinase inhibitor sorafenib. We have already shown that sorafenib inhibits growth of dedifferentiated thyroid carcinoma cell lines with and without BRAF mutation. The purpose of this study was to analyze the molecular effects of sorafenib on cell signalling and cell cycle.
Methods
In thyroid carcinoma cell lines (anaplastic, dedifferentiated follicular and papillary), we analyzed inhibition of signaling molecules of the MAP kinase pathways and of members of the receptor tyrosine kinases after sorafenib treatment by means of phospho-specific antibodies. Flow cytometric cell cycle analyses were performed to investigate cellular effects of sorafenib. Sorafenib was kindly provided by Bayer HealthCare.
Results
After sorafenib treatment, a rapid inhibition of members of the Map-kinase family including MEK1 and MEK2, Erk1 and Erk2 and p38 Map-kinases was achieved. Additionally, members of the Jnk kinase family were inhibited, while the Akt pathway was unaffected. We also detected inhibitory effect of sorafenib on receptor tyrosine kinases like PDGF receptor alpha and beta and VEGF receptors, while EGF receptors and other erbB receptors were not affected. After 4 to 8 h of sorafenib treatment, cells showed apoptosis induction. Cell cycle analyses revealed a sorafenib-dependent arrest mainly in the S phase of the cell cycle in the remaining living cells, while the G0/G1 peak was diminished.
Conclusion
The BRAF- and multi-targeted kinase inhibitor sorafenib showed various inhibitory effects on intracellular signaling pathways in thyroid carcinoma cells and caused cell cycle arrest and apoptosis induction. Since sorafenib was effective in all thyroid carcinoma cell lines independently of the presence of an activating BRAF-mutation, this drug may be a new therapeutic option for dedifferentiated thyroid cancer.

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A rare case of hiperandrogenism bilateral Leydig cell tumor of the ovary

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Background
The androgen-secreting tumors constitute fewer than 1% of ovarian tumors. Leydig cell tumor is one of the most common of this type of lesion and it is usually benign, small and unilateral.
Case report
A 67-year-old women was referred to the Endocrine clinic due to hirsutism (score 22 Ferriman–Gallwey) and male type alopecia with 3 years of evolution and progressive worsening. Biochemically she had high levels of serum testosterone – 662 ng/dl ($N<62$ ng/dl). Transvaginal ultrassonography, and abdomen–pelvic CT scan didn't show any signs of adrenal or ovarian tumors. Bilateral oophorectomy was performed and histopathologic exam revealed bilateral Leydig cell tumors, measuring 1 cm each. Four months after surgery, the patient had a marked improvement of the signs (score 3 Ferriman–Gallwey) and normalized testosterone levels (35.9 ng/dl).
Discussion
Androgen-secreting tumors are rare, but they should be excluded in cases of rapid onset of virilization and elevated androgen levels. Tumors are frequently undetectable by imaging techniques because of their small dimensions. Exploratory surgery by a skilled team is frequently necessary and is often the best treatment for the majority of these cases. We present a rare case of bilateral Leydig cell tumor. Only five cases have been reported in the literature. The clinical history and the elevated levels of testosterone had suggested the presence of an androgen-producing tumor. Attempting to the fact that our patient was in a post-menopausal stage, we decided that the appropriate treatment would be bilateral oophorectomy. The histopathologic disclosed the diagnosis and allowed the patient's cure.

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Distinct catecholamine phenotypes in hereditary pheochromocytoma

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This study examined whether different forms of hereditary pheochromocytoma are characterized by different catecholamine phenotypes and whether this is reflected by differences in plasma concentrations of normetanephrine, metanephrine and methoxytyramine – the respective O-methylated metabolites of norepinephrine, epinephrine and dopamine. Subjects included 154 patients with hereditary pheochromocytoma, 72 with tumors associated with von Hippel–Lindau (VHL) syndrome, 39 with multiple endocrine neoplasia type 2 (MEN 2), 4 with neurofibromatosis type 1 (NF1), 34 with mutations of the succinate

dehydrogenase type B (SDHB) gene and 5 of the succinate dehydrogenase type D (SDHD) gene. Plasma and urinary catecholamines and metabolites, and tumor tissue catecholamines in a subset of patients, were measured by HPLC. Relative proportions of norepinephrine, epinephrine and dopamine in tumor tissue were closely matched by relative increases of plasma O-methylated metabolites, but not by those of the parent catecholamines. Patients with tumors due to MEN 2 or NF1 had increases in both plasma metanephrine and normetanephrine, whereas those with mutations of VHL, SDHD and SDHB genes showed increases mainly in plasma normetanephrine. Plasma levels of methoxytyramine were increased in 65% of patients with tumors due to mutations of the SDHB gene, but were otherwise generally normal. The study establishes that differences in tumor catecholamine phenotypes can be accurately assessed using measurements of plasma O-methylated metabolites and that these phenotypes differ markedly among patients with different hereditary forms of pheochromocytoma. This information may be useful in determining relative likelihoods of different disease-causing mutations in patients who otherwise have no clinical stigmata or family history consistent with a hereditary syndrome.

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VDR gene polymorphisms in patients with differentiated thyroid cancer

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Background/aim

Vitamin D receptor (VDR) expression has been shown to be upregulated in several tumors and is thought to represent an important endogenous response to tumor progression. Therefore, in order to evaluate the role of VDR-gene and of the 25(OH)-Vitamin D_3 in thyroid cancer we analysed four polymorphisms in patients with thyroid cancer and healthy controls.

Patients and methods

Patients ($n=136$; 84 females and 52 males) with differentiated thyroid cancer (follicular or papillary) and healthy controls ($n=210$; 99 females and 111 males) were genotyped for the ApaI (rs7975232), BsmI (rs154410), TaqI (rs731236) and Fok I (rs10735820) polymorphisms within the VDR-gene in the German population by the PCR-RFLP method. In addition, the 25(OH)-Vitamin D_3 serum levels in patients were measured using RIA.

Results

The genotype aa of the ApaI polymorphism was significantly less frequent (5.1 vs 18.6%; $P<0.0003$) while heterozygosity was more frequent in patients than in controls (62.5 vs 45.2%). No association between BsmI, TaqI and Fok I and thyroid cancer was observed. Furthermore, 74% of the patients showed low serum levels of 25(OH)-Vitamin D_3 (<20 ng/ml).

Conclusion

Both genotypes (Apa I polymorphism) within the VDR-gene and low serum levels of 25(OH)-Vitamin D_3 appear to be associated with differentiated thyroid cancer Germans. Nevertheless, additional work is necessary to define the extended genotype of VDR and other loci of the vitamin D system and their impact on the 25(OH)-Vitamin D_3 levels as well as their possible clinical applications.

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ERCC1 expression in adrenocortical carcinoma: relationship with baseline characteristics and response to platinum-based chemotherapy

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Adrenocortical carcinoma (ACC) is a malignant tumor with poor prognosis and no established therapy in advanced stage. Cisplatin is the most frequently used cytotoxic drug, but even combined with doxorubicin and etoposid, the response rate is $<50\%$. Recently, it has been demonstrated that the excision repair cross complementing group 1 (ERCC1) plays a relevant role in the DNA repairing process, particularly in the correction of platinum-induced DNA adducts. Accordingly, ERCC1 expression predicted prognosis in patients with different types of cancers when treated with platinum-compounds. In this study, we evaluated the expression of ERCC1 by immunohistochemistry in ACC and correlated it with clinical outcome. We evaluated 170 adrenal tumor samples spotted on three tissue microarrays, comprising 15 benign tumors (5 inactive, 5 cortisol producing and 5 aldosterone producing adenomas) and 155 ACCs. ERCC1 protein was highly expressed in 14 benign tumors (93%) and only in 71 ACCs (46%, $P<0.005$). No differences in baseline clinical or histological parameters were found between ACC patients with high and low ERCC1 staining. No impact of ERCC1 expression on overall or disease-free survival was observed in patients who did not receive platinum-based chemotherapy. However, ERCC1 expression was associated with clinical outcome in patients treated with platinum-based regimens ($n=36$). Two of 17 'positive' patients had a partial response to treatment, while 4 of the 19 'negative' patients experienced a partial ($n=3$) or a complete response ($n=1$). In univariate analysis, ERCC1 expression was the only significant predictor of survival after first platinum administration (median survival: 7 months in 'positive' versus 17 months in 'negative' patients, HR: 2.1, 95% CI: 1.1–5.5, $P<0.05$). In conclusion, ERCC1 expression may be a novel prognostic factor for predicting survival in ACC patients treated with platinum-based chemotherapy and may provide critical information for individualized treatment.

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Leptin exerts apoptotic effects and regulates androgen receptors in human prostate cancer cells

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Introduction

Prostate cancer (PCa) progression is known to depend on various hormones and growth factors, but their role and underlying molecular mechanisms remain poorly understood. We recently presented preliminary findings indicating that leptin causes a greater level of activation of the JAK2/STAT3 and MAPK (ERK1/2) pathways, as well as transactivation of HER2, in androgen-sensitive LNCaP cells than in androgen-insensitive PC3 and DU145 human PCa cell lines. We and others were previously unable to demonstrate proliferative effects of leptin in LNCaP cells. We now studied the effects of leptin on LNCaP cell apoptotic proteins stimulated by starvation medium-induced stress via the intrinsic pathway, as well as on androgen receptor (AR) protein expression.

Results

Leptin (0.1–10 ng/ml; 6–72 h) caused clear apoptotic effects, seen as an increase in the downstream apoptotic effector caspase 3 protein expression ($\leq$2.3-fold; av. $\pm$ S.E.M.: 1.86 ± 0.09) and in cleaved (inactivated) poly-(ADP-ribose)-polymerase (cPARP89), an enzyme normally responsible for DNA repair and a downstream substrate of caspase 3 ($\leq$2.1-fold; av. $\pm$ S.E.M.: 1.60 ± 0.26). Leptin also caused increased expression of AR protein ($\leq$2.6-fold; av. $\pm$ S.E.M.: 1.96 ± 0.34). All of these leptin effects were mostly maximal at 0.1–1.0 ng/ml and already at 6 h and generally still maintained at 10 ng/ml and for up to 72 h or gradually reduced (caspase 3) from 24 to 72 h. With view to understanding the mechanism of these leptin effects, we studied the effects of the JAK2 inhibitor AG490 on leptin-induced signalling and apoptotic and AR proteins. In a preliminary experiment, leptin-induced pJAK2 and pAKT (10 min) were clearly inhibited by AG490, while the inhibitor had lesser effects on leptin-induced apoptotic and AR proteins (6 h).

Conclusions
Clearly, further studies with other kinase inhibitors will be needed to delineate the mechanism of leptin-induced apoptosis in human PCa and its relationship with the effects of leptin on AR. These studies are expected to provide new insights into the possible role of leptin, presumably together with other hormones and growth factors, in the progression of human PCa or its delay, and may provide a basis for discovery of new drugs for therapy of PCa.

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Long-term survival after surgical treatment in patients of adrenocortical carcinoma

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Adrenocortical carcinoma is a rare tumor characterized aggressive growth and poor prognosis. This tumor requires the complex way of treatment, where basic method is surgery.
The aim of this study was to evaluate the long-term outcome and role of surgery in patients with adrenocortical carcinoma.
Methods
From 1998 to 2007, 44 patients were operated for adrenocortical carcinoma (14 cortisol-secreting tumors, 1 virilizing tumor and 29 nonfunctioning tumors). There were 28 women and 16 men, with a medium age 56.3 years (range, 21–72). The mean tumor diameter was 8.4 cm (range, 3–21), weight – 301.2 g (range, 21–2000). The pathological tumor's stage was I (4 patients), II (8 patients), III (23 patients) and IV (9 patients).
Results
Complete resection was performed in 41 patients. Among them, three patients had combined resection *en bloc* with the adjacent organs (kidney, spleen and pancreas). Three patients underwent liver segmentectomy and one patient lung resection. Incomplete tumor's resection was performed in 3 patients. The mean follow-up was 48.2 months (range, 2–96). Twenty-six patients are still alive and 18 were died. The 5-year overall survival, calculated by the Kaplan–Meier method, was 58.5%. The 5-year overall survival rate for patients at stage I, II, III and IV was 98.7, 82.3, 56.7 and 0%, respectively. Local recurrence or metastases were observed in 13 patients. The mean disease-free survival was 26.4 months (range, 6–42). The 3-year disease-free survival was 40.5%. No significant differences in 3-year disease-free survival rates and tumor's stage were revealed.
Conclusion
Radical surgery with a complete resection of the primary tumor, adjacent organs and, when feasible, solitary metastases offer the best prospects for long-term survival.

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Parathyroid-hormone related- Peptide and PTHrP receptor type 1 are expressed in human adrenocortical carcinoma and regulate cell proliferation and apoptosis in H295R an adrenocortical-derived cell line

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Adrenocortical tumor (ACT) is a rare, heterogeneous malignancy whose pathogenesis is unclear. The oncoprotein PTHrP, found in many common tumors, can regulate their growth in an autocrine/paracrine fashion through the receptor PTH-R1. Little is known about the role of PTHrP in ACT. We monitored the synthesis of PTHrP and PTH-R1 in a series of 25 ACT: 12 adrenocortical carcinoma (ACC), 13 adrenocortical adenoma (ACA), and investigated the effects of PTHrP (1–34) on H295R cells derived from adrenocortical carcinoma. PTH-R1 mRNA and proteins were detected by RT-PCR and western blotting in all the ACT samples and in H295R cells. There was no significant difference between their concentrations in the ACT. PTHrP mRNA was assayed by quantitative real-time PCR. It was detected in 90% of ACC, and in 10% of ACA. There was a positive correlation between the usual pronostic factors (tumors size and weight, MacFarlane stages, Weiss Score), and steroid hormone production. Tissue-specific PTHrP protein processing was shown by western blotting. Immunohistochemical staining revealed numerous, dense foci of PTHrP-containing cells in ACC, but few positive cells in ACA or normal tissue. PTHrP stimulated the growth of H295R cells, while specific anti-PTHrP antibody and PTHrP-R1 antagonist both enhanced their apoptosis. PTHrP did indeed activate the cell cycle in H295R cells and released the G1/S checkpoint, resulting in a redistribution of cells in the S and G2/M phases. PTHrP activated both adenylate cyclase/protein kinase A and the intracellular calcium /protein kinase C pathways via PTHrP-R1. The active synthesis of PTHrP is linked to a poor prognosis in ACC, where it may act as an autocrine/paracrinre factor in tumor growth and malignancy.

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Prognostic factors in localized adrenocortical carcinoma (ACC) after complete resection

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Even after complete resection, patients with ACC have a high risk of relapse, and adjuvant treatment with mitotane is frequently recommended. Although mitotane has significant efficacy in this setting, it is associated with a wide range of side effects. As survival is highly variable, prognostic factors are of great interest to better guide adjuvant therapy after radical resection.
We analyzed clinical and histopathological data of patients without distant metastases registered with the German ACC registry ($n=291$). As patients with histologically confirmed complete resection (R0; $n=162$) have a significant better 5-year survival than patients with R1 resection ($n=14$)(68% (95%-CI: 59–78%) vs 36% (13–58); $P<0.01$) or with uncertain resection status (Rx; $n=72$)(51% (37–65%); $P=0.06$), we only included patients with R0 resection in the detailed analysis.
In univariate analysis, the following factors were indicators of poor prognosis for disease-free and overall survival (DFS/OS): tumor size >8 cm, positive lymph nodes, tumor(thrombus) in v.cava, Ki67 $>10\%$, absence of atypical nuclei or atypical mitoses, mitoses $>5/50$ HPF, spongiocytic tumor cells $<25\%$, vascular invasion. Multivariate analysis demonstrated improved or impaired DFS ($P<0.05$) for the following factors: atypical nuclei (hazard ratio 0.39 (95% CI: 0.27–0.55)), atypical mitosis (HR 0.43 (0.31–0.59)), mitoses $>5/50$HPF (HR 2.3 (1.6–3.2)), advanced age (HR 1.02 (1.01–1.02)), positive lymph nodes (HR 3.2 (1.9–5.1)), and vascular invasion (HR 1.8 (1.3–2.4)). Furthermore, spongiocytic tumor cells $<25\%$ (HR 6.9 (2.6–18.1)), tumor (thrombus) in the v.cava (HR 2.8 (1.9–4.0)), and positive lymph nodes (HR 3.5 (2.3–5.3)) were associated with decreased overall survival. Using these factors, it is possible to calculate prognostic indices. A patient without any of these adverse risk factors has a 5-year disease-free survival rate of $>90\%$.
In conclusion, we have identified important prognostic factors in ACC patients with R0 resection based on clinical and histological parameters. These factors may help to guide adjuvant therapy in ACC.

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Multi-center, observational study on Sandostatin LAR treatment patients with acromegaly in Poland: preliminary report

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Aim
The aim of multi-center, observational study was to assess the outcome of the treatment with somatostatin analogue octreotide-LAR (O-LAR) patients with acromegaly.
Material
Material consisted of 360 patients (60% women) aged 18–84 years (mean 45.5; S.D. ±12.9) with active acromegaly. Prior to the inclusion 172 patients underwent unsuccessful neurosurgery.
Methods
Observation was planned for 1 year. O-LAR monthly dose was adjusted to 10–30 mg accordingly to IGF-1 levels. Assessments of clinical symptoms and GH/IGF-1 was performed quarterly. Visual field and pituitary MR was performed initially, and after 12 months.
Results
Observation completed 330 patients from 29 centers in Poland, mainly from Warsaw (84), Poznań (51) and Silesian region (45). Mean period of symptomatic disease prior to diagnosis was 8.8 years (1–24 S.D. ±4.9). Concomitant diseases was hypertension (64.2%), diabetes mellitus (20.3%) and heart failure (8.3%). Goiter was present in 59.2% and hyperthyroidism in 8.6% cases. Prior to O-LAR treatment pituitary macroadenoma was shown in 62%. Initially, mean GH concentration was 19.5 μg/l (S.D. ±35; 1.0–449 μg/l) and decreased significantly during treatment to 8.4 μg/l (S.D. ±11.5) after 3 months, and finally to 5.6 μg/l (S.D. ±7.9), $P<0.0001$. Parallelly, mean IGF-1 concentration was 894.5 μg/l (S.D. ±350; 209–2461 μg/l) and decreased to 573.5 μg/l (S.D. ±311) ($P<0.001$), and finally to 457 μg/l (S.D. ±262) ($P<0.001$). IGF-1 normalization was achieved in 61.6% patients. Pituitary tumor volume decreased significantly (>25%) in 57% of patients. Treatment led to significant reduction of acromegaly symptoms: soft tissues edema was present initially in 95%, after treatment – in 47%, hyperhydrosis in 90 and 42%, respectively, headaches 80–49%, arthralgia 85–55%, weakness 69–43%, carpal tunnel syndrome 18–4%, paresthesiae 43–39%, sleep apnoea 19–8%, galactorrhoea 6–1%. Adverse effects were mild and mainly affected gastrointestinal system (diarrhea in 15% and during treatment fell to 5%, abdominal pain 8% fell to 4%, and nausea affected 8% and fell to 5%. No therapy discontinuation occurred. This is the largest observational study to assess O-LAR efficacy in acromegaly reported. Results proves, high efficacy of medical therapy with somatostatin analogue in terms of clinical and biochemical improvement. Therapy is safe and well-tolerated.

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A study on investigations for localizing insulinoma

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We studied the practice of investigations for diagnosing and localising insulinoma in our hospital. We looked at the number of investigations performed before exact localisation of insulinoma.
We identified 5 cases that were confirmed on clinical, biochemical or histopathological bases and were recorded on the histopathology and IT database as 'insulinoma' from 1986 to 2004. We looked retrospectively at the case notes of these cases.
All 5 cases had blood tests for insulin and c-peptide during a 72 h fast. Three out of 5 had abdominal ultrasound and this was normal in 2 and inconclusive in 1. All had abdominal CT scan and was normal in 3. One CT scan was normal 8 years after symptoms onset and a repeat CT, after 15 years of symptoms, localized a mass. One CT localized the lesion quite close to the exact location as found on surgery. Angiogram, portal venous sampling and gut hormone profile were performed in 2 patients. One patient had endoscopic ultrasound which localized insulinoma accurately. Two patients had pancreatic MRI which were inconclusive. Four patients had surgical exploration and all of these had intra operative ultrasound (IOUS). IOUS localized lesions only in 1 of 4 patients. Octreotide scan was done in 2 and both were normal.
These findings suggest that none of the tests performed were able to localize insulinoma accurately all the time. We do not seem to have any consensus on any protocol for specific investigations to localize these disabling tumours and we performed a number of different imaging techniques. There is a need for drawing joint guidelines by physicians and surgeons for localizing these tumours accurately.

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Epidemiology of pituitary tumours in Iceland 1955–2007: a Nationwide Study

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Pituitary tumours may be more prevalent than previously appreciated. Although this is thought to relate mostly to greater utilisation of imaging techniques in recent years, evidence suggests that the increased prevalence also applies to clinically important tumours.
We have created a nationwide registry of pituitary tumours occurring in Iceland for the last 55 years. We have examined medical, surgical, pathology and imaging records at all hospitals as well as private practices in Iceland. We also scrutinized records of out of hospital imaging facilities and the Icelandic Cancer Registry.
A total of 312 individuals have been identified during this period, 119 men and 193 women ($P<0.03$). Overall, the median age at diagnosis was 41.9 years (range 3–88 years) being 35.2 years for women and 53.3 years for men. The most common tumour types were prolactinomas in 35% and non-functioning adenomas in 26%. The overall age standardized incidence was 2.9/100.000 population for women and 1.8/100.000 for men while the prevalence in December 2006 was 82/100.000 population. For women the incidence increased from 0.1/100.000 to 6.3/100.000 comparing the first and last quarter of the study period while comparable figures for men were 0.4/100.000 and 3.6/100.000. The increase in incidence observed was clearly associated with the introduction of modern imaging technology to Iceland. However, 84% of the individuals did have symptoms or signs attributable to the tumour at diagnosis.
The increased incidence and current prevalence of pituitary tumours in Iceland is comparable to recent studies from elsewhere. The observation that the majority of the patients had symptoms or signs attributable to the tumour highlights the importance of general physicians being aware of this condition.

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Effects of pasireotide on bronchial carcinoids in primary culture

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Bronchopulmonary endocrine tumors represent 25–30% of lung neoplasms. Surgery provides good survival for differentiated tumors (typical and atypical carcinoids), but is not useful for aggressive poorly differentiated forms. Somatostatin (SRIF) analogs can be used as medical therapy, prolonging patient survival. However, the compounds employed so far did not display antiproliferative effects. Recently, a new stable SRIF analog, pasireotide (SOM230), which activates SSTR1, 2, 3, and 5, has been developed. Our study aimed at clarifying whether SOM230 might affect cell viability of bronchial carcinoids *in vitro*. We assessed SRIF receptor expression pattern, both at mRNA and protein level, as well as the *in vitro* effects of SOM230 on cell viability. Sixteen brochial carcinoids (13 typical and 3 atypical carcinoids) were examined by RT-PCR and by microscopy immunofluorescence for expression of Chromogranin A (CgA) and SSTRs. In addition, primary cultures were tested with SOM230 *in vitro*. All samples expressed CgA, while SSTR1 was expressed in 14 samples, SSTR2 and SSTR4 in 12, SSTR3 in 5, and SSTR5 in 7 samples. Treatment with SOM230 did not significantly modify cell viability, but was capable of blocking the proliferative effects of Forskolin, a potent stimulator of cyclic AMP (cAMP) pathway. This effect was apparent in primary cultures from carcinoid tumors expressing SSTR1 or SSTR2, as well as in tumors not expressing SSTR3 or SSTR5. Our data demonstrate that cAMP pathway activation up-regulates bronchial carcinoid cell proliferation, which can be inhibited by SOM230 in selected cases. Indeed, our data suggest that this effect is mediated by SSTR1 or SSTR2, and not by SSTR3 or SSTR5. Since SSTR1 and 2 are the most represented among bronchial carcinoids, our data support a possible role for SOM230 as a new tool for medical therapy of these tumors.

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The prognostic factors in adrenocortical carcinoma

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Adrenocortical carcinoma (ACC) is a rare neoplasm with poor prognosis. Patients present signs of hormone excess: virilisation, Cushing's syndrome or only enlarged abdominal mass. Incidentally ACC can be also detected in the ultrasonography. Some of 'non-hypersecretory' ACCs can produce non-bioactive hormones steroid precursors or not very big amount of them and sometimes patients present subclinical Cushing's symptoms. Surgery and adjuvant radiotherapy and chemotherapy with Mitotane is the treatment of choice.
Aim
The aim of the study was to analyse clinical features, hormonal test results and prognosis in patients with secreting and non- secreting adrenal cancers.
Patients
The course of disease of 22 patients: 6 men and 16 women median age 45.5 year was analysed. Clinical examination, the imaging studies and hormonal assays were performed. Seventeen patients underwent surgical treatment. Mitotane was administered in 16 patients.
Results
Virilization was diagnosed in 3 patients, overt or subclinical Cushing's syndrome in 12 patients. Fourteen patients were classified as the I-st or II-nd stage, 8 as the III rd or IV th stage. The total and the asymptomatic survival time was longer in non-secreting patients than in secreting (53.86 vs 32.6 and 53.42 vs 13.9 months respectively). The secretory tumours were bigger than non-secretory (10.34 vs 6.15 cm). Due to ACC progress 10 patients died among which only one had non-secretory tumour. The mean time of observation was 13.7 months. Three patients with advanced disease and 9 patients with complete remission are still alive. In all patients with long-term remission, the mean diameter of tumour was 5.7 cm, and the mean time of observation is 76.6 months.
Conclusion
The poor prognostic factors in ACC are: size of tumour, presence of local and distant metastases and hormonal activity. Chemotherapy with mitotane prolong life of patients, but is less effective in advanced disease with excessive steroid hormone secretion.

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Cushing's syndrome due to a pigmented nodular adrenocortical disease and a acromegaly corresponding to a Carney complex (CNC)

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The CNC is a dominantly syndrome, characterized by spotty skin pigmentation, endocrine overactivity and myxomas (Carney & Young 1992), associated with lentigines and blue naevi; the disease links to 17q22-24, and its mutations have been identified in the genes PRKAR1α. The most common endocrine manifestations affect two o more endocrine glands, including acromegaly, thyroid and testicular tumours and ACTH-independent Cushing's syndrome due to primary pigmented nodular adrenocortical disease (PPNAD). There are around 160 cases reported.
We describe the case of a 52-year-old menopausal woman who complained for pain in the right flank, but the anamnesis reflected symptoms of mild hypercortisolism (hypertension, troncular over weight, asthenia, strengthless…) and a spotty skin; a Cushing's syndrome was confirmed: lost of the circadian rhythm of cortisol (26.3 mcgr/dl pm 24.5 mcgr/dl), high free urine cortisol (135 mcgr/24 h), and no suppression of the cortisol levels after 1 (259 mcgr/dl) and 8 mg (285 mcgr/dl) of Dexametasone. A 2.6 cm lesion in the left adrenal was described in the abdominal scanner and also a 8.5 cm mass in the left kidney. She was operated and the histopathology reported a pigmented nodular hyperplasia of the adrenal and an angiomiolipoma. The follow-up verified a cortisol deficiency; although there was and improvement of the symptoms, she still complained about asthenia and malaise with pain in the joints. Acromegaly was diagnosed after the pituitary study, with confirmation of elevated IGF-1 levels (482 ng/dl) and no suppression of the GH levels after OGTT (peak of GH 7 ng/dl) and an image of global growth of the pituitary in the MRI. The response to the treatment with somatostatin analogs (Sandostatin 20 mg/28 - days) was good, clinically and the analysis. Breast tumour, cardiac myxoma, thyroid disease and colonic cancer were ruled out, after the images studies. The genetic study of mutations in the PRKAR1α was request.
We conclude that the hyperplasias that cause Cushing's syndrome more frequently than was tought, and they are associated with other diseases, including CNC. Genetics defects of phophodiesterases might be a frequent cause of adrenal and other tumours.

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Self-observations of total life situation in patients with Acromegalia

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Background
Acromegalia is an infrequent chronic disease requiring lifelong control. A total of about 350 Danish patients have acromegalia. It is difficult for this patient group to find information on coping strategies in fellow patients.
There are numerous international quantitative studies on the symptoms in acromegalia patients; no qualitative studies on self-observations as a way to exchange experiences and optimize nursing in connection with hospitalisations.
The purpose was to clarify patients' self-observations on:

- What it was like to live with a disease for many years before it was diagnosed and treated.
- Nursing is in connection with hospitalisations.
- Patients' outlook/perception of the future.

Method
The theoretical approach was the operative constructivism by Niklas Luhmann. The qualitative interviews were based on the method of Steiner Kvale. From April to December 2007, 37 patients were interviewed in connection with hospitalisations at Aarhus University Hospital, Denmark. The semi-structured interview guide was based on Katie Eriksson's theories about disease, nursing and life suffering. The method of analysis was meaning condensation. Analyses will be made in January 2008.
Preliminary results
Some patients see themselves as healthy, but most patients experience discomfort:

- They are unfit for work
- They have problems structuring everyday life
- They have social problems
- They worry about the future

Anticipated conclusion:
The results can be used for

- Patient information
- Optimization of nursing
- Patient education to enable patients to manage complications at an early stage.

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Mitotane induces a concentration-dependent impairment of platelet aggregation in patients with adrenocortical carcinoma

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Standard treatment of adrenocortical carcinoma (ACC) comprises adrenolytic therapy with mitotane. Prolongation of bleeding time has previously been observed based on a series of 7 patients (Haak *et al.* 1991). As patients with ACC frequently undergo surgery for local recurrence or metastases, we have studied the effect of mitotane on coagulation in 44 patients with ACC before and/or during treatment with mitotane (total sample size $n=62$).
Platelet aggregation was performed with standard light transmission aggregometry using platelet rich plasma. Major components of the extrinsic and intrinsic coagulation cascade pathways were determined by routine coagulation testing using the Dade Behring BCS analyzer system or manual ELISA testing. *In vitro* bleeding time was detected as PFA-100 measurement.
During mitotane therapy, a successive decrease in platelet aggregation occurred that was closely correlated with mitotane levels. This effect was most pronounced for ADP-induced platelet aggregation (Pearson $r=0.77$; $P<0.001$) and induction with collagen ($r=0.47$; $P=0.002$), but less so for challenge with epinephrine ($r=0.25$; $P=0.1$), and ristocetin ($r=0.18$; $P=0.2$). Platelet counts, *in vitro* bleeding time, global plasmatic coagulation and von Willebrand parameters remained unaffected. With mitotane levels above 10 mg/l, 18 out of 19 patients had pathologic ADP-induced platelet aggregation.

In conclusion, in a large series of patients with ACC we show impaired agonist-induced platelet aggregation strongly dependent on plasma mitotane levels. Routine *in vitro* bleeding time is not suitable to detect this platelet defect and to assess bleeding risk. ADP-induced platelet aggregometry testing prior to surgery is recommended to determine mitotane-induced bleeding risk. Further studies should investigate the potential role of prophylactic administration of DDAVP to improve platelet function.

P321

One hundred minimally invasive parathyroidectomies without intra-operative localisation and PTH monitoring

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Background
Minimally invasive parathyroidectomy (MIP) for primary hyperparathyroidism is routinely performed in many centres. Various preoperative and intraoperative localisation techniques are used along with intraoperative PTH monitoring (IOPTH).
Results
We report the results of 110 consecutive patients presenting to our unit with a diagnosis of primary hyperparathyroidism from January 2004 until November 2007. All patients had sestamibi scintigraphy (MIBI) as the primary investigation for preoperative localisation. One hundred patients had a positive scan showing a single gland and underwent MIP by a single surgeon without any further pre or intraoperative localisation studies and without IOPTH monitoring. The remaining 10 patients underwent further localisation studies and bilateral conventional neck exploration and were not included in the study. In 95 patients, a single abnormal gland concordant with the localisation scan was identified and excised. In 5 patients, no obvious abnormal gland was identified; they were converted to bilateral neck explorations. In one among these, an abnormal gland was found on the opposite side to the scan report and was excised. In remaining 4, 3½ glands were excised. Primary outcome measure of normocalcaemia at 6 months was achieved in 95/100 (95%). Of the 5 patients who remained hypercalcaemic, a second abnormal gland was identified on repeat MIBI scan in 1 patient and successfully removed by further uncomplicated surgery. Two of the 4 remaining patients were subsequently diagnosed as suffering with familial hypocalciuric hypercalcaemia and 2 with parathyroid hyperplasia and treated conservatively.
Conclusion
In the majority of patients with primary hyperparathyroidism and a positive MIBI scan, MIP can be safely undertaken without any further pre or intraoperative localisation and without IOPTH monitoring.

P322

Effect of Ginkgo biloba extract supplementation on genotoxic damage after thyroid remnant ablation by ^{131}I

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Background
Radioiodine (^{131}I) therapy is performed in patients with differentiated thyroid cancer (DTC), either for thyroid remnant ablation or treating distant metastasis. Although ^{131}I therapy is generally considered safe, a genotoxic damage has been demonstrated both *in vivo* and *in vitro*.
Aim
To evaluate the possible effect of Ginkgo biloba extract (EGb 761) supplementation on the time-course (up to 120 days) of clastogenic factors (CFs) and micronuclei (MN) appearance in lymphocytes from patients with DTC after thyroid remnant ablation, in a double-blind, placebo controlled study.
Methods
Nineteen patients with DTC (12 F, aged 30–68 years; administered ^{131}I activity: 2.96–5.50 GBq) were randomly assigned to EGb 761, 120 mg/day for 1 month ($n=9$, 6 F) or placebo ($n=10$, 6 F). Blood was taken at various intervals after ^{131}I therapy (from 7 to 120 days), for the evaluation of the time-course of CFs and MN appearance in peripheral lymphocytes.
Results
In the placebo group, MN significantly increased after ^{131}I therapy ($P<0.001$, ANOVA for repeated measures), peaking at 7th day ($P<0.005$) and slowly declining thereafter. Conversely, in EGb 761-treated patients, a slight not significant increase of MN without a peak was observed. Therefore, mean MN increment was significantly higher in placebo- than in EGb 761-treated patients ($P<0.01$). Moreover, an early (7th day, $P<0.005$) and sustained ($P<0.01$, ANOVA for repeated measures) MN increase induced by CFs was observed in the placebo group while, in EGb 761-treated patients it never reached the statistical significance. Again, mean MN increase induced by CFs was significantly higher in placebo- than in EGb 761-treated patients ($P<0.05$). Thyroid remnant resulted ablated in all the patients.
Conclusions
Although ^{131}I therapy is essentially safe, our data encourage for the use of Ginkgo biloba extract to prevent possible harmful genetic effects, particularly in patients with metastasis who require repeated radioiodine treatments.

P323

Neuroendocrine tumours (NETs): one centre experience

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Background
NETs are rare tumours arising from dispersed neuroendocrine system. Nevertheless, their estimated prevalence increased lately, mostly due to progress in imaging, biochemical and histopathological diagnostics.
The aim of the study was to present the characteristics of the NETs patient surveyed in our Endocrinology Department since 2000.
Material and methods
One hundred and fifty-eight patients (males – 42.4%, females – 57.6%) aged 18–82 years were included in the analysis. Medullary thyroid cancer, small cell lung cancer and pheochromocytoma patients were excluded.
Results
According to WHO NETs classification patient in stage Ia constituted 2.5%, Ib-38.6%, II-57%, III-1.3%, IV-0.6% of the analyzed group. The most common localization of the primary tumour was pancreas (34.2%). About 18.4% of studied NETs arose from foregut, 25.3% from midgut (including 5% tumours from appendix) and 17.1% from hingut. Five percent of disseminated NETs had unknown primary origin. Among the NETs there were such rare as epiglottis and gallbladder carcinoids. Distant metastases were found in 29% of the patients, which may be attributed to the relatively high proportion of indolent gastric NETs, insulinomas, and early diagnosed rectal carcinoids. Functioning NETs constituted 41.1% of the group. About 77.2% of the patients were treated surgically, 9.5% received systemic chemotherapy, 19.6% – radioactive peptide therapy. About 5.1% of the study group died – most often due to complications of ectopic Cushing's syndrome.
Conclusions
Modern and widely accessible diagnostic procedures (particularly endoscopy and endoscopic surgery) result in increased number of early recognized NETs. It implies the possibility of radical surgical treatment, although due to indolent behavior of NETs all patient should be periodically screen for distant metastases. Relatively low mortality among NETs patient, even with disseminated disease, may be attributed not only to the tumour biology, but also to the new methods of treatment (particularly radiopeptide therapy), which are able to stabilize the disease and offer control of its symptoms.

P324

Abnormalities in glucose tolerance in acromegalic patients

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Background
Acromegaly is characterized by disabling symptoms and relevant comorbidities. Insulin resistance, leading to glucose intolerance is one of the most important contributory factor to the cardiovascular mortality in acromegaly.
Aim
To assess the impairments of glucose homeostasis in acromegalic patients and find association between activity of the disease and the severity of glucose intolerance.
Patients and methods
In this retrospective study, we analyzed records of 220 patients (138 females – 62.27% and 83 males – 37.73%) with untreated acromegaly diagnosed at our

Department in the years 1994–2006. Diagnosis of active acromegaly was established on the basis of elevated plasma GH above 1 ug/l during the 75 g OGTT and elevated plasma IGF-1 above normal range for age and gender. The patients with overt diabetes mellitus did not undergone the test. The abnormalities in plasma glucose concentrations were categorized according to WHO criteria.
Results
In the studied group, the mean age at the moment of diagnosis was 46.28 ± 13.82 years. The duration of the disease since the onset of the first symptoms was 7.50 ± 4.97 years.
Normoglycemia existed in 45.52% of acromegalic patients. Among glucose tolerance abnormalities we found impaired fasting glucose (IFG) in 20%, impaired glucose tolerance (IGT) in 5.52%, both IFG and IGT in 6.90% and overt diabetes mellitus in 22.07%. There was no statistically significant difference in basal plasma GH and IGF-1 concentrations between normoglycemic patients and with impairments in glucose tolerance. The groups statistically significantly differed when the age of diagnosis was concerned ($P=0.038$). There was no significant correlation between fasting plasma glucose and GH, IGF-1 concentrations.
Conclusions
The frequency of diabetes was four times higher in acromegalics than in general Polish population which should urge clinicians to treat this disease not only to reduce growth factors, but also to maintain the favorable effects on metabolism and metabolism-related mortality.

P325

Mutations in the ret proto-oncogene in Romanian patients with multiple endocrine neoplasia type 2

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Multiple endocrine neoplasia (MEN) is characterized by the occurrence of tumors involving two or more endocrine glands; MEN 2 is defined by medullary thyroid carcinoma in association with phaeochromocytoma and appears in several clinical variants, which may be inherited as autosomal dominant syndromes. Mutational analysis of RET protooncogene has been use in the diagnosis and management of patients and families with MEN 2 variants.
Aim
In this study, we retrospectively analyzed 20 patients with MEN 2 from 14 different families for relevance of specific mutations in the RET proto-oncogene.
Materials and methods
Genomic DNA was extracted from peripheral leukocytes using a Promega blood minikit and two fragments covering the exons 10 and 11 of the RET gene were amplified by polymerase chain reaction using specific oligonucleotide primers (exon 10: 5′-GCCTATGCTTGCGACACCAGTTG-3′, exon 11: 5′-CATGAGG-CAGAGCATACGCA-3′) and the following protocol; 95CX 10 min followed by 30 cycles 95CX 1 min, 64CX 1 min, 62CX 30 s and a final extension at 72C for 7 min. The resulting fragments were sequenced directly with the Big Dye R Terminator v 3.1 cycle sequencing kit using a ABI Prism 310 Genetic Analyser.
Results
In 13 of 20 examined MEN 2 cases, missense mutation of codon 634 from exon 11 of the RET proto-oncogene were detected. Nine of the MEN 2 samples proved to have an identical base sequence change TGC-TGG resulting in the substitution of Cys with Trp, two of the samples have the change TGC-GGC resulting in the substitution of Cys with Gly and other two samples have the change TGC-CGC resulting in the substitution of Cys with Arg. We cannot detect mutations in exon 10 or 11 of RET protooncogenes in 7 patients with specific tumors associations.
Conclusion
We conclude that, in all families with MEN 2, mutational analysis of the RET proto-oncogene should be performed both to identify specific mutations and to prove gene carrier status for MEN 2.

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Is the early postoperative hormonal assessment a helpful predictor of the long term remission in secreting pituitary adenomas after transsphenoidal adenomectomy?

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Introduction
Transsphenoidal adenomectomy is the treatment of choice in secreting pituitary adenomas with the symptoms of Cushing's disease, acromegaly as well as few cases of *Prolactinoma*, which – due to resistance to pharmacological treatment – require surgery. The efficacy of selective adenomectomy ranges from 60 to 95%. It is the highest in patients with well-visualized by MRI microadenomas, lower in macroadenomas and the lowest when the surgery was performed without any changes in imaging techniques.
Due to complicated diagnostic procedures and the necessity of prolonged postoperative observation it is necessary to prove which hormonal laboratory findings drawn shortly after pituitary surgery could foresee the lasting remission. The early identification of a high risk of recurrence would allow to perform an immediate re-operation that increases the chance of cure during the same hospital stay.
We have examined 40 patients with secreting pituitary adenomas being prepared at our site to surgical treatment and followed up for the next 12 months. All patients were operated by the same neurosurgeon performing over 100 transsphenoidal surgeries per year.
All patients had blood draws for serum cortisol, GH or PRL performed on the first postoperative day and additionally FSH, LH, TSH. The same laboratory tests were taken after 6, 12, 24 weeks and 12 months after surgery.
The results of our study indicate that subnormal (<2.0 ng/ml) serum cortisol levels in the first postoperative day in CD can foresee lasting remission. In case of acromegaly, the low levels (<1.0 ng/ml) of serum GH can predict long-term remission. In patients suffering from *Prolactinoma*, we perform surgery only in tumors resistant to pharmacological treatment. The early results seem to be promising.
Conclusion
The early results show a correlation between low serum cortisol, GH and PRL levels in the 1st day after surgery and long term remission. It simplifies postoperative diagnostic procedures. In case of CD, we expect that the cut off point will be lower than 5 ng/ml – previously obtained in other studies. In addition, the early postoperative cortisol assessment allows to predict post-operative function of the pituitary–adrenal axis.

P327

Prognostic value of 100 mcg s.c. octreotide test (SHort Octreotide Test – SHOT) for prediction of medical treatment outcome in patients with acromegaly

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Background
Neurosurgery is treatment of choice in patients with GH-secreting pituitary adenoma. However, in about 50% of cases surgery is ineffective or contra-indicated. Long-acting somatostatin analogues are possible, although expensive therapeutic alternative. Generally, 60–70% of patients with active disease responds to such medical treatment.
Aim
Aim of this study was to identify factors influencing medical treatment outcome and to determine if testing injection of 100 mcg octreotide s.c. (SHort Octreotide Test – SHOT) may be used as predictive tool.
Material
Material consisted of 137 patients (88 F and 49 M) aged 17–83 years (mean 49.6) with active acromegaly, treated 2001–2005 in one center. Neurosurgery was primary therapy in 48 cases.
Methods
SHOT: GH assessed in 30 min intervals during 2 h acute 100 mcg octreotide s.c. test. Treatment initiation: GH and IGF-1 concentrations were determined at 1, 3, 7, 14 and the 28 day following test dose 20 mg of OCT-LAR i.m. Prolonged treatment: GH and IGF-1 assessments were performed quarterly during 2–5 years of medical treatment. Imaging (MR) was performed yearly and KNOSP scale was assessed.
Results
Significant reduction of GH concentration (by more than 75%) was achieved in 48 out of 137 cases (35%) SHOT suppressed GH below 2 ng/ml, but in 93 (68%) GH reduction by more than 75% was achieved. Administration of the first OCT-LAR dose suppressed GH below 2 ng/ml in 44 patients (32%) and in 93 (68%) by more than 75%, whereas the IGF-1 levels dropped to age–sex reference range in 39 cases (28%). Most pronounced reduction of GH level (mean drop from 26.2 to 10.8 ng/ml) was registered at the 14th day following the OCT-LAR. During prolonged treatment, GH and IGF-1 levels decreased slowly during first year, and then remained stable (mean 8.3 ng/ml S.D. 5.7 and 497 ng/ml S.D. 312 for GH and IGF-1, respectively). IGF-1 normalization were achieved in 76 (55%).

Reduction of GH levels in SHOT tightly correlated with the GH level during long-term treatment ($R=0.68$, $P<0.001$). Assessment of GH and IGF-1 during first month of therapy has no additional value. Reduction of GH below 2 ng/ml defines cohort of most probable IGF-1 normalization (sensitivity (sen.) 96% and specificity (spe.) 95.5%), whereas by >75% precisely predicts good outcome of long term therapy (sen. 97% and spe. 70%). Other, independent factors of good outcome is symptoms duration <10 years (sen. 72% spe. 65% and KNOSP grade <3 sen. 63% spe. 48%).
Conclusions
SHOT is effective in identification of cohort with favourable prognosis of pharmacological treatment efficacy.

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Cushing syndrome with an atypical evolution
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A 72-year-old depressed woman complained about fatigue, weight gain (20 kilos/2 years), severe and recent hypertension, easy bruises, and venous insufficiency. Whereas she was treated with six antihypertensive drugs, she had hypokaliemia (2.6 mM). Cardiac ultrasounds showed myocardial hypertrophy. A Cushing syndrome was suspected. UFC was increased (400 mM/d), cortisol cycle disrupted and a standard DXM test showed no cortisol suppression (775 nM/l). ACTH levels were consistently low. A 4 cm round mass with calcifications, discovered by CT scan in the left adrenal gland, was removed by laparoscopy. Pathological summary was 5 cm benign adrenal tumour (Weiss score 2). The patient was treated with 15 mg hydrocortisone. Nine months later, she had gained 3 more kilos with biological signs favouring osteoporosis. UFC was 1280 nM/d without DXM suppression (cortisol 604 nM/l) and kaliemia 3.2. A new CT scan visualized a polylobular mass in the same surgical area and several 'nodules' near pancreas cauda, spleen and around spleen vein. After a ketoconazole short treatment, about twenty small nodules from 1 mm to 2.5 cm were surgically removed in the epiploon, near the pancreas cauda and the spleen, similar to the main tumour (extemporaneous examination), apparently.
Pathological results indicated that nodules limited by fibrosis were mostly agglomerates of large cells with 9 mitoses/50 large power fields and necrosis. Some foci were different with apoptosis, necrosis and oncocytes: 8 mitoses/10 hpf (Ki 67 score 10–15%). There was no vascular invasion. Pathological diagnosis was adrenocortical carcinoma. The previous report was reviewed and noticed a 3 Weiss score (tumour of uncertain malignancy). The patient is treated with mitotane, hydrocortisone and fludrocortisone. Her condition is currently stable with some side effects of mitotane.
This observation underlines the remaining difficulties of pathological exam of endocrine tumours even when they are performed by skilled pathologists.

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Parathyroid carcinoma treated with cinacalcet: a preliminary report
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A 61-year-old man was hospitalized for paresthesias and renal insufficiency (creatinine clearance 24 ml/min) with proteinuria. Type 2 diabetes was diagnosed in 2003 and hypertension in 2007. Hypercalcemia (4.6 mM) with normal phosphorus level 1.23 mM was discovered with very high PTH levels (863 ng/l; $N<78$). MIBI scintigraphy localized a right inferior parathyroid tumour. No obvious sign favouring MEN1 was observed and no familial case was reported. After a short pamidronate treatment and hydration, surgery allowed to remove the tumour and a parathyroid carcinoma was diagnosed. Post surgery PTH levels decreased to about 100 ng/l. Two months later, a thyroid right lobectomy with lymph nodes dissection was performed. No invasion was found. One month later, PTH was still high 191 ng/l with serum calcium concentration 2.18 mM and phosphorus level 0.78 mM. No hungry bone syndrome was observed and 25-OH Vit D was in the low-normal range 40 nM. SestaMIBI scintigraphy and cervical ultrasonography were normal. Eight months later PTH was very high 419 ng/l with hypercalcemia 3.3 mM and low phosphorus levels 0.52. An 18 Fluoro-FDG PET scan was normal with no fixation on bones or lungs. Creatinine clearance was 54 ml/min. After bisphosphonate and hydration without major effect (calcemia 2.88 mM), cinacalcet was begun with titration: 30 mg BID one week, 60 mg BID three weeks. Calcium levels were slightly decreased at 3 mM with low phosphorus levels: 0.38; 0.45; 0.61 mM. Cinacalcet dosage is now increased to 90 mg BID and is presently well tolerated (no nausea or vomiting noticed). Treatment titration is still going on; a new sestaMIBI scintigraphy is planned. Though emergent, Cinacalcet seems promising to control hypercalcemia in parathyroid carcinoma. Actualization of our data will be given with genetic results of HRPT2.

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Ovarian serous and mucinous tumors of low malignant potential: patterns of stromal invasion
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Purpose
The purpose of this study was to evaluate the histologic spectrum of stromal–epithelial patterns of invasion in ovarian serous and mucinous tumors of low malignant potential.
Materials and methods
We retrospectively analyzed 31 cases of borderline ovarian serous and mucinous tumors diagnosed of the Pathology Department of County Hospital Timisoara in a period of 5 years, between 2003 and 2007. Epithelial architecture, the patterns of stromal invasion, number of invasive foci, size of individual invasive foci, and the extent of the nondestructive foci were reviewed.
Results
Five patterns of invasion were identified: (1) cell clusters and individual, detached eosinophilic cell (27/31, 87%), (2) simple individual papillae and simple branching papillae (8/31, 25.8%), (3) cribriform pattern (6/31, 19.35%), (4) complex branching micropapillae (2/31, 6.45%), (5) inverted macropapillae (5/31, 16.12%). Most cases contained a mixture of stromal–epithelial patterns.
Conclusions
The distinction between ovarian serous and mucinous tumors of low malignant potential and low-grade serous and mucinous carcinoma can be quite difficult because former type present a variety of stromal–epithelial alteration. The most common stromal–epithelial pattern of invasion on this study was the individual intra-stromal eosinophilic cell and cell cluster pattern.

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Novel *MEN1* germline mutations and clinical features in Greek patients with multiple endocrine neoplasia type 1
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Introduction
Multiple endocrine neoplasia type 1 (MEN1) is an autosomal dominant hereditary disorder, associated with mutations of the *MEN1* gene, characterised by the combined occurrence of tumours of the parathyroid glands, the pancreatic islet cells and the anterior pituitary.
Aim
To identify *MEN1* gene mutations and characterize clinical manifestations in Greek patients with MEN1.
Patients and methods
We studied 4 unrelated index patients (one male and 3 females, age range 30–65 years). Twenty relatives, 8 affected (4 males and 4 females, age range 30–71 years) and 12 unaffected (7 males and 5 females, age range 22–74 years) and 100 control subjects, were also studied. DNA extraction, polymerase chain reaction

(PCR) and sequencing the MEN1 exons 2–10 and exon/intron boundaries were performed according to standard procedures.
Results
We identified novel *MEN1* gene mutations in 3 out of 4 index patients (75%) and in 8 affected (100%) as well as in 2 unaffected (16.6%) relatives. Novel mutations included a frameshift mutation in exon 4 (c.794-795insG) at codon 229 resulting in the premature termination of menin synthesis at codon 231 (Family 1); a missense mutation in exon 4 (c.886T>C) which substitutes leucine with proline at codon 259 (L259P) (Family 2); a frameshift mutation in exon 8 (c.1270_1280dupAGGAGCGGCCG) involving codons 387–390, resulting in truncation of the menin protein at codon 447 (Family 3). We also found that all our 13 *MEN1* carriers (100%) had clinical symptoms and/or biochemical abnormalities. In the fourth index patient, only a common polymorphism (D418D) was detected.
Conclusions
These data indicate the high prevalence of novel *MEN1* gene mutations among MEN1 patients and the absence of genotype/phenotype correlation. Mutational analysis for *MEN1* identifies healthy carriers who are at a high risk of developing tumours and helps in the clinical management of the patients and their families.

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Ghrelin and its receptor are present in an ectopic ACTH lung neuroendocrine tumour causing Cushing's syndrome: potential pathophysiological implications

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Ghrelin is a 28-aa peptide originally isolated from stomach but present also in many tissues, including hypothalamus and pituitary, where it stimulates growth hormone (GH) release through the ghrelin/GH secretagogue receptor (GHS-R). Ghrelin also increases food intake and adiposity and could play a key integrative role in the endocrine–metabolic interface. Although its primary pituitary cell target are somatotropes, ghrelin also modulates other pituitary cell types, specially in certain pathologies. Indeed, we recently showed that human pituitary adenomas causing Cushing's disease express both ghrelin and GHS-R. Moreover, ghrelin was co-stored within ACTH secretory granules, and increased free cytosolic calcium ($[Ca^{2+}]_i$) and presumably ACTH release via GHS-R. These results provided a cellular basis for the exaggerated ACTH response to ghrelin in Cushing's disease and suggested an association of the ghrelin/GHS-R system with the pathophysiology of corticotropinomas. Because Cushing's syndrome is frequently caused by ectopic ACTH secretion from non-pituitary neuroendocrine tumours, specially lung carcinoids, we explored the possible presence of ghrelin/GHS-R system in these tumours. Specifically, a 70-year-old woman was diagnosed of a lung carcinoid tumour with ectopic ACTH secretion. Freshly isolated tumour lung pieces were processed for: (a) immunomicroscopy; (b) measurement of ghrelin-induced changes in $[Ca^{2+}]_i$ and secretory activity in single living cells; and (c) RT-PCR expression analysis. As expected, 97% cells from the ectopic lung carcinoid tumour showed ACTH immunofluorescence. RT-PCR confirmed ACTH (POMC) expression and further revealed that the lung carcinoid tissue also express ghrelin and GHS-R1a. Moreover, ghrelin (1 μM) stimulated $[Ca^{2+}]_i$ in 80% of cells ($n=20$), and increased the rate of incorporation of the fluorescent dye FM-5-95, suggesting increased hormone release. Taken together, our results demonstrate that, as in pituitary corticotropinomas, ghrelin and GHS-R1a are functionally present in an ectopic corticotropinoma causing Cushing's syndrome, and could therefore provide an autocrine mechanisms involved in the pathophysiology of these tumours.

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Deficits in the clinical management of patients with adrenocortical carcinoma in Germany

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Adrenocortical carcinoma (ACC) is a rare disease with poor prognosis. Accordingly, in many cases, the attending doctors have no previous experience with the disease. The aim of our study was to evaluate the quality of care in a large number of patients with ACC in Germany. Data from 263 adult patients of the German ACC registry were analyzed with regard to the following parameters: time to diagnosis, hormonal assessment, imaging, histopathological documentation, follow-up. The time from first symptoms to diagnosis varied widely, e.g. in patients with Cushing's syndrome from several weeks to 36 months (median 8.5 months). Preoperative diagnostic work up was frequently insufficient: In the majority of patients no (16%) or only incomplete (46%) hormonal investigations were performed, putting the patient at unnecessary risk and impairing future follow-up. Furthermore, in 47% no thoracic CT was performed. Histopathological assessment by the respective local pathologists proved frequently not reliable: during the last two years, the diagnosis of ACC was revised by the reference pathologist of the registry in 7 cases. In addition, resection status was not given in the histopathological report in 15.4% of patients, although this information is of key importance for treatment and prognosis. We detected deficits also in follow-up: due to the high rate of recurrence in ACC, regular restaging every 3 months is recommended. However, in a third of patients a first postoperative follow-up was performed only after more than 6 months. In conclusion, we have identified significant deficits in the care of patients with ACC in Germany. Based on recommendations of the European adrenal network ENSAT only 33% of the patients underwent sufficient hormonal assessment, staging, and postoperative follow-up. Close and early interaction of physicians with specialized centres will be of key importance to improve the care of patients with this rare disease.

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The cAMP analog 8CL-cAMP enhances PKA RIIb, triggers apoptosis and blocks cells at S and G2 cell cycle phases after 4 days of treatment in the human adrenocortical cells H295R.

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Various alterations of the cyclic AMP (cAMP) signalling cascade has been observed in adrenocortical tumors. Changes of the PKA regulatory subunits expression in tumors may positively or negatively regulate proliferation. 8CL-cAMP, a site selective cAMP analogue, induces growth inhibition in a variety of human cancer cell lines. The aim of the study was to determine if 8CL-cAMP acts on adrenocortical carcinoma H295R cell growth.
We have determined the involvement of PKA, PKA R subunits in this activity and studied the mechanisms of action on cell cycle molecule targets and cell cycle distribution. Cells were incubated for 96 h or 7days with 8CL-cAMP (10^{-4} M). Cell viability and proliferation was evaluated by MTT assay and BrdU incorporation. Apoptosis was investigated by annexin FITC assay. Western blotting was performed, for PKA subunits, cyclin and cyclin dependent kinase protein expression. Cell cycle distribution was analysed by flow cytometry.
8CL-cAMP induces PKA activation, modulates the expression of PKA regulatory subunits by increasing the type II isozymes. The cAMP analogue reduces cell proliferation and enhances apoptosis in the H295R cells. These effects were associated with the accumulation of cells at S and G2 phases. This is consistent with the accumulation of cyclin A in the nucleus and cyclin B both in the cytosol and the nucleus. Cells blocked from progression through M phase by nocodazole (1 μM, 18 h) or G1/S phase by aphidicollin (2.5 μM, 24 h), revealed that addition of 8CL-cAMP resulted in a delay in S/G2 transition and a blockage in G2/M chekpoint.
These results imply that cAMP/PKA RIIb pathway regulates S and G2/M phase progression in H295R. Activation of PKA RIIb holoenzyme may be responsible for the growth arrest, by altering the cell cycle regulator pattern and the induction of apoptosis.

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Hyperprolactinaemia: different clinical expression in childhood

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Hyperprolactinaemia is the most common disturbance in the pituitary gland function. Different physiological and patological conditions could influnce prolactin (PRL) secretion. Prolactin secreting tumors (micro and macroprolactinomas) are rare in children and adolescents (estimated incidence is 1 per milion). Functional diversity of prolactin action is responsable for different initial clinical expression of hyperprolactinaemia.

We investigated causes of hyperprolactinaemia in 11 children and adolescents (6 females and 5 males), aged from 1.5 to 17.5 years with different clinical expression at admission. Children with primary hypothyroidism, jatrogenic hyperprolactinamia and PCOs adolescents were excluded. Four patients had short stature or growth deceleration, the same number was clinicaly obese, two adolescent girls had secondary amenorrhoea, two girls had premature thelarche and gynaecomastia and hypogonadism were indication for endocrinologic examination of two adolescent boys. Vasculitis and obesity and recidivant herpes viral infections and obesity were present in two school children. Hyperprolactinaemia was found also in the youngest girl with multiple ovarial cysts.

We documented very high PRL level in PRL profile in all patients (mean 2100 mU/l). MRI of pituitary was indicated and revealed 4 microprolactinomas, 1 congenital hypophyseal cyst and 1 tumor of the hypothalamus.

Children with hyperprolactinaemia expressed a wide variety of initial clinical presentations. The most common were growth and puberty disorders and obesity. PRL determination should be included in investigation protocols of obese and short children as prolactinoma, although rare, is nevertheless a likely disease, even in childhood.

P336

$G_{12/13}$-dependent growth of small cell lung cancer cells *in vitro* and *in vivo*

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The malignant phenotype of small cell lung cancer (SCLC) cells critically relies on the autocrine stimulation of $G_{12/13}$- and $G_{q/11}$-coupled neuropeptide receptors. By this means, $G_{12/13}$- and $G_{q/11}$-dependent signalling pathways are constitutively activated in these cells. While $G_{q/11}$-dependent signalling has been shown to promote proliferation of SCLC cells, the role of signalling pathways via $G_{12/13}$ is still elusive. Thus, we employed shRNA-mediated down-regulation of $G\alpha_{12}$, $G\alpha_{13}$, or both, in human SCLC cell lines. The use of a lentiviral expression system rather than non-viral transfection resulted in robust, specific, and stable knockdown of both target proteins. This decrease in $G\alpha_{12}$ or $G\alpha_{13}$ levels markedly reduced proliferation in SCLC cell lines in normal culture medium as well as colony formation in semi-solid medium. However, double targeting of both $G\alpha_{12}$ and $G\alpha_{13}$ did not exert a synergistic anti-proliferative effect *in vitro*. In a subcutaneous tumor xenograft mouse model, the downregulation of $G\alpha_{12}$ or $G\alpha_{13}$ resulted in decreased tumor growth, which was due to reduced tumor cell proliferation. More importantly, double knock-down of $G\alpha_{12}$ and $G\alpha_{13}$ completely abolished tumorigenicity in mice. Thus, intact $G_{12/13}$ signalling is a prerequisite for proliferation of SCLC cells *in vitro* and tumorigenicity *in vivo*.

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High prevalence of pituitary adenomas: a cross-sectional study in the city of Banbury (Oxfordshire)

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Background

Pituitary adenomas (PA) are considered very rare conditions, with an estimated prevalence of 25 cases/100 000 inhabitants. However, community-based studies on the prevalence of PA are currently scant.

Aim

To ascertain the prevalence of PA and the characteristics of the patients diagnosed with them in a large population of inhabitants.

Methods

A survey on the GP surgeries of Banbury (Oxfordshire, UK) covering 89 334 inhabitants was carried out following approval from the Local Research Ethics Committee. Finally, 14 out of 16 approached GP Surgeries agreed to participate allowing the inclusion of 81 149 inhabitants. Patients were initially identified through IT search at the Surgeries using relevant search terms. The notes of the selected subjects were scrutinized to confirm the diagnosis.

Results

We found 64 cases (43 females; median age at diagnosis (MAG) 37 years (range 16–74)), and the prevalence was 78.87 cases/100 000 inhabitants. The series comprised 36 prolactinomas (56%), 7 patients with acromegaly (11%), 11 non-functioning adenomas (NFA) (17%), 1 Cushing's disease (1.6%) and 9 pituitary masses of unknown functional status (UF) (14%). The median symptomatic period before diagnosis in non-apoplectic PA was 1.7 years (48 patients, range 0.5–15 years). MAG and sex distribution were: prolactinoma (89% female, MAG 32 years), NFA (18% female, MAG 54 years), acromegaly (43% female, 47 years), Cushing's disease (0% female, MAG 57 years), UF (66% female, MAG 39 years).

Conclusions

This is the first cross-sectional, community study on the epidemiology of PA performed in a large population of the United Kingdom. Our prevalence is three times higher than previous estimates, and prolonged symptomatic periods before diagnosis were common. PA affects patients at a young, economically active population. Prolactinomas are far more common in females, while acromegaly and NFA are more common in males. Increased awareness of these treatable conditions is important to reduce diagnostic delay.

P338

Gastric NETs: new diagnostic and therapeutic approach

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The incidence of GNT appears to be increasing, what can be explained by the increased detection caused by the common use of the endoscopy and the pervasive use of acid suppressive therapy leading to enterochromatofine like cells proliferation. There are numerous new diagnostic/therapeutic GNT methods in use like: EUS, SRS, somatostatin therapy and 90Y/177Lu-DOTA-TATE radiotherapy.

Materials and methods

In 1998–2007, 25 patients were diagnosed with the hist. path. confirmed GNT (mean age – 59 ± 12; 19 F, 5 M). In all patients gastroscopy, CT/MRI, EUS 99Tc-EDDA/HYNIC-Octerotate scintigraphy, chromogranin A serum level, clinical manifestation of the disease and type and efficacy of the therapy were assessed.

Results

Among 25 GC patients, in 65% type I in 5% type II and in 30% type III was diagnosed. During 4 years of the observation 6 patients with dissemination died (2 patients- type I, 1- type II and 3 -type III). The best detective value was found for the 99Tc-EDDA/HYNIC-Octerotate scintigraphy both for the primary and the metastatic lesions. In all patients, the increased level of chromogranin A was found (55.8 ± 98 U/l; *n*: 2–18 U/l), maximum value in patients with dissemination (max. 295 U/l). In most cases partial/total gastric resection was performed. However, in 3 patients with type I GNT treated only with the somatostatin a complete endoscopic remission was observed in 1 case, and partial remission in 2 cases.

Conclusion

As the number of GNT is increasing the extensive diagnostic and therapeutic methods development are needed. However, the endoscopic or surgical gastric resection are still a basic treatment, the use of somatostatin in type 1, somatostatin and 90Y/177Lu-DOTA-TATE radiotherapy in nonoperative, disseminated cases seems to be very promising. Due to the different clinical course of the disease it seems that the treatment should be individually tailored to reach the best and optimal effect.

P339

Fenotypes in patients with Y791F mutation of RET protooncogene

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Mutation Y791F of *RET* protooncogene is a well known mutation so far described in families with FMTC and familial pheochromocytoma in one family. It activates the receptor in a monomeric form. Here we present eight unrelated families with the same mutation but different fenotype expression.
Patients and methods
In last 20 years, we analyzed 216 patients with MTC (age range: 3–75 years, 45.0 mean). Genetic testing for mutation in *RET* proto-oncogene was performed by direct double-strand sequencing of the PCR products obtained after amplification of genomic DNAs.
Results
Of 216 patients with MTC, in 48 (22.3%) familial form of disease was found. In 23 patients (34.3%) from 8 non-related families germ-line mutation was found in c791 (Y791F). Only four had MTC and they were index cases in their families. The first two patients were diagnosed, operated and cured at the age of 33 and 42, respectively. Metastatic MTCs were diagnosed at the age of 53 in the third patient and she died at the age of 62. The fourth patient was diagnosed with metastatic MTC at the age of 62. No one had associated diseases. Nineteen patients were carriers (age range: 10–68, mean 35.7) with no evidence of the diseases but 6 of them (31.6%) had kidney development malformations. One patient had kidney hypoplasia and PHPT. She was negative for MEN1 mutations.
Conclusion
The frequency of inherited MTC in our population is in concordance with other publications, but the frequency of c791 mutation is higher than in other studies. Phenotype varies from no evidence of oncogenic activity to overt disease. While more aggressive disease has been described in patients with coexistent L769L polymorphism, the occurrence of kidney malformations may reflect the insufficient RET protein expression despite oncogenic mutation during the embryogenesis or the coexistence of other disease.

P340

Insulin sensitivity in a patient with gastric ghrelinoma

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Ghrelin is a somatotrophic, orexigenic and adipogenic hormone that has an important homeostatic role by linking regulatory systems for growth and energy balance. Ghrelin- secreting neuroendocrine tumors are rare, but we had the opportunity to study a 71 years old female patient with a gastric ghrelinoma. The patient was submitted to gastroenterological examination after having episodes of diarrhea, and small subepithelial polyp (5 mm) was revealed on gastroscopy. Pathohystological analysis after endoscopic biopsy proved it to be a well-differentiated neuroendocrine tumor (Ki 67–1%) with immunostaining positive for general neuroendocrine markers (CgA and synaptophysin $+++$) as well as for ghrelin ($+++$). The patient was otherwise in good health condition, was slightly obese (BMI-27.4 kg/m^2) and had no clinical signs of acromegaly. Serum ghrelin level was elevated. It has been previously shown in gastrectomized patients that short-term ghrelin administration during hyperinsulinemic euglycemic clamp induces a transient increase in GH levels, a decrease in IGF1 level, and a decrease in C-peptide level, thus having a negative influence on insulin secretion and glucose consumption. Our patient had normal IGF1 level for age and gender (155 ng/ml, ref. 64–188), but her basal GH level was elevated (3.9 ng/ml), with no signs of GH-producing pituitary tumor. Her basal insulin level was normal (17.6 mmol/l), with marked increase during glucose tolerance test ((300 mmol/). We performed a hyperinsulinemic euglycemic clamp and found significantly reduced M value (2.83 mg/kg bw per min). We conclude that chronic hyperghrelinemia may induce insulin and GH resistance.

P341

Association of genetic variants of somatostatin receptor 5 with acromegaly

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Full gene coding sequence of somatostatin receptor 5 and 2000 bp of upstream region was estimated using direct sequencing in 28 patients with acromegaly and 97 controls. Total 19 polymorphisms were identified (SNP1-19) and possible haplotypes were reconstructed. From all polymorphisms found silent substitution SNP15 was significantly associated with acromegaly as compared with control group ($P=0.005$). Another substitution SNP17 involves the amino acid change from proline at position 335 to leucine that was less frequent in acromegalic patients compared to controls ($P=0.02$). Moreover, two patients homozygous for SNP15 were resistant to somatostatin analog sandostatin as characterized by elevated GH and IGF-I levels as well as recurrent surgical removal of adenomas. These data suggest the role of SSTR5 genetic variants in progression of acromegaly as well as possible identification of genetic marker of resistance to somatostatin treatment. Present study has been approved by Latvian Central Committee of Medical Ethics.

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Cushing syndrome: cycling around diagnosis

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Introduction
Cyclic Cushing syndrome (CCS) involves rhythmic fluctuations in ACTH secretion resulting in a cyclic variation of adrenal steroid production.
Case presentation
A 37-year-old woman was referred to our Pituitary Clinic in 1999, for evaluation of galactorrhea and irregular menses that had started four months ago. She also complained of depression, facial hirsutism, alopecia and progressive obesity. Blood pressure was normal. Endocrinological evaluation revealed prolactin blood level elevated twice and a half the reference value, low gonadotrofins blood values, and urinary free cortisol (UFC) of 166.1 μg/24 h (reference: 28.5–214). The cerebral magnetic resonance imaging suggested a non-functioning pituitary macroadenoma without optic quiasm compression. Two years later, tumor dimensions were unchanged and UFC remained normal (50.6) but without cortisol circadian rhythm. One year later, she was admitted with diabetic ketoacidosis and 1 month later, insulin was suspended because of frequent hypoglycemias. Shortly thereafter, she was readmitted because of vomiting, metabolic alkalosis and hypokalemia. Four months later, basal UFC was 387.8 and ACTH was 128 pg/ml (reference: <46), with a paradoxical response to the high dose dexamethasone supression test. Despite medical advice, she left the country before pituitary surgery. Three years later, she returned to our Clinic and normal UFC and ACTH were observed. Surgery was performed in 2007 and immunochemistry revealed diffuse positive ACTH tumor staining.
Discussion
One of the proposed explanations for the mechanism behind the CCS of pituitary origin is the occurrence of spontaneous, episodic haemorrhage in the tumor with temporary damage of the actively secreting cells. CCS should be strongly suspected in patients with signs or symptoms of hypercortisolism but normal cortisol levels and paradoxical responses to the dexamethasone test.

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Raloxifene induces growth arrest and apoptosis in prostate cell lines expressing both ERα and β

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Raloxifene (RAL) is a selective estrogen receptor (ER) modulator (SERM) proposed for chemoprevention of breast cancer and osteoporosis. SERMs exert agonist–antagonist effects depending on tissue or ERs expressed. RAL induces apoptosis in both androgen-dependent and independent cell lines, suggesting a selective activation of ERβ and prevalent antagonist effect on ERα in prostate cells (PC). In this study we evaluated effects of estradiol (E2) and RAL on epithelial prostate cell growth, using two *in vitro* models: the androgen-dependent epithelial PC line EPN, that expresses both ERs, and a stabilized PC line derived from prostate cancer (PCaECS) that lacks both ERs. Semiconfluent starved cultures were treated with E2 or RAL (10^{-9}–10^{-6} M) or solvent. Cells were harvested 48 h after the treatment and stained with propidium iodide for flow cytometry analysis

by FACS calibre or analyzed by TUNEL assay for *in situ* DNA fragmentation, or recovered for western-blot analysis. Activation of MAPK was also evaluated in short term experiments. An increase of apoptosis and of S and G2-M cell cycle phase inhibition with G0/G1 arrest was observed in EPN after E2 (10^{-9} M) or RAL ($10^{-6.8}$ M). Percent of apoptosis and cell growth inhibition were also significant when 10^{-9} M RAL was used ($P<0.05$ vs control). Bcl-2 protein levels in cell extract were significantly reduced by E2 (10^{-9} M) and RAL (10^{-6} to 10^{-9} M); PAR-4 levels increased in EPN after E2 or RAL. In PCaECS, there was no effect on cell growth control after E2 or RAL. RAL induced a rapid and transient phosphorylation of ERK in EPN and sustained effects on PCaECS.
Conclusions
Pharmacological concentrations of RAL that acts as a partial ERβ agonist and ERα antagonist are able to inhibit cell growth in EPN, but result inefficient in PCaECS lacking both ERs.

P344

Pituitary adenomas: the reality of Braga

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Introduction
There are limited data on the incidence and prevalence of pituitary adenomas (PA). Although classically considered rare, systematic assessments from autopsy and radiological studies have shown a prevalence of 17% in the general population.
Objective
We describe the epidemiologic of PA in Braga, the third most populous district of Portugal (860 000 inhabitants, 2673 km^2), over the last 20 years.
Methods
We reviewed the clinical files and the laboratorial, radiological and pathological data of all the patients with pituitary tumors diagnosed in our Pituitary Reference Center. The prevalence and incidence were expressed as number of cases/million people and number of cases/million people per year, respectively.
Results
A total of 160 patients (123 female (F) and 62 male (M)) with prolactinomas – PR-(59 F; 16 M), non-functioning PA – NFPA-(18 F; 25 M), GH-secreting (25 F; 8 M), or corticotrope adenoma – CA-(8 F and 1 M), were characterized. The mean age at diagnosis was 41.7 ± 17.3 years (range: 14–85 years) being for PR 32.7 years, for NFPA 53.2 years, for GH 45.6 years, and for corticotrope adenoma 49.3 years. The prevalence of PA was 186, being for prolactinomas 87.2, for NFPA 50.0, for GH-secreting 38.4, and for CA 10.5. In the last 10 years, the mean annual incidence of PA was 16.4, being for PA 7.1, for NFPA 4.5, for GH-secreting 3.7, and for CA 1. We identified 66.3% of macroadenomas, which corresponded to 46.7% of prolactinomas, 100% of non-functioning PA, 67% of GH-secreting, and 55.6% of corticotrope adenoma.
Conclusions
The frequency of different PA types, the age of presentation and female predominance verified in our district are similar to literature. The growing awareness and the diagnosis improvement may account for the apparent increase in PA incidence, over time.

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Deregulated Wnt/β-catenin signaling in breast cancer: the LRP5Δ receptor

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Background
Aberrant accumulation of cytoplasmic/nuclear β-catenin frequently occurs in breast cancers and breast cancer cell lines. Deregulated expression of Wnt ligands, sFRP1 and Dvl1, but no inactivating or activating mutations in Wnt signaling components have so far been reported. We have recently described an internally truncated LRP5 receptor (LRP5Δ) in 91% of hyperparathyroid tumors, and showed that LRP5Δ was strongly implicated in the aberrant accumulation of active ß-catenin in these tumors (Björklund *et al. PLoS Med* 2007).
Objectives and methods
We have initially analyzed a small number of primary breast carcinomas (19) and several breast cancer cell lines, as well as normal breast tissue for expression of LRP5Δ. Further, we have investigated function of LRP5Δ in the Wnt/β-catenin signaling pathway and in maintaining tumor cell growth both *in vitro* and in an *in vivo* xenograft mice model.
Results
We have found expression of the LRP5Δ receptor in 16 of 19 primary breast carcinomas and in several breast cancer cell lines, including MCF-7. No expression of LRP5Δ was observed in normal breast tissues. Experiments on MCF-7 cells will be presented, strongly supporting a fundamental role of the LRP5Δ receptor in deregulated Wnt/ß-catenin signaling and tumor growth in transplanted SCID mice.

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p107Rbl1 is an haploinsufficient tumor suppressor in mouse pituitary

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The pocket protein family members, pRb, p107 and p130, are negative regulators of the cell cycle. But, while the role of pRB as a tumor suppressor has been extensively established, genetic inactivation of p107 or p130 is uncommon in tumors. Previous studies have shown that p130 cooperates with the CDK inhibitor p27Kip1 restricting proliferation of mouse intermediate lobe pituitary cells. Accordingly double knockout mice show higher incidence of pituitary tumors than single KO animals. Because of functional redundancy between p130 and p107, to study contributions of p107 as a tumor suppressor we have generated p107+/−; p130−/− mice and combined them with p27 inactivation (5/6KO strain).
We found that reduction of p107 gene dosage caused a significative reduction in tumor-free survival time. As previously described, p27 deficient mice developed adenomas almost exclusively in the pituitary and adrenal medulla, while p130 mutation collaborated to increase the frequency of pituitary and adrenal tumors. In this context, 5/6KO mice showed accelerated appearance of pituitary tumors, while the pheocromocytomas, mostly unilateral in p27 null and DKO mice, where bilateral with almost complete penetrance. Strikingly, microscopic examination of pituitary tumors revealed that in the case of 5/6KO mice tumors were clearly invasive and showed features of aggressive carcinomas.
When we studied the expression of cell cycle regulators, we found than wildtype intermediate lobe has undetectable amounts of p107. This finding was confirmed in extracts from p27 deficient adenomas. Nevertheless, p107 protein was detected in p130 null animals, suggesting a mechanism of compensatory expression. Moreover, by both RT-PCR and immunoblot we found that p107 was still expressed in tumors arising in the 5/6KO mice, indicating that p107 can act as a haploinsufficient tumor suppressor in mouse pituitary.

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Immunohistochemical evaluation of ghrelin expression in somatotroph and non-functioning pituitary tumours

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Introduction
Ghrelin is known to strongly stimulate growth hormone release from the pituitary. It was also found to be produced locally in hypothalamus and anterior pituitary and in different types of pituitary adenomas. It's been suggested that ghrelin synthesized locally in hypothalamo–pituitary area or within pituitary tumours can be an important factor contributing to pituitary tumorogenesis.
Aim
The aim of the study was the immunohistochemical assessment of ghrelin expression in somatotroph and non-functioning pituitary tumours and the comparison of the presence of ghrelin in somatotropinomas treated and untreated preoperatively with a long acting somatostatin analogue.
Materials and methods
The studied material consisted of somatotroph and non-functioning pituitary tumours tissues obtained during neurosurgical removal of the tumour. There were somatotropinomas obtained from patients treated with a somatostatin analogue (octreotide LAR) before the surgery and not treated (paraffin sections collected in

the past when SS analogues were not routinely used in presurgical farmacotherapy). As a control for immunohistochemical analysis, the mucous membranes of the stomach (obtained during gastrectomy due to stomach neoplasms) as well as normal pituitary tissues (obtained during autopsy) were used. Tumour tissue samples were placed in formaline and then in paraffine. Immunohistochemical study was done with the use of rabbit polyclonal antibodies against human ghrelin 1:400 in TBS (Phoenix Pharmaceuticals, Inc).
Results
Immunohistochemical analysis confirmed that ghrelin is present in non-functioning pituitary tumours and somatotroph adenomas, even after the treatment with octreotide LAR. Characteristic staining was observed in the cytoplasm of cells, in some of them predominantly in perinuclear area. The expression of ghrelin was also shown in normal pituitary, it was, however, restricted only to few cells.
Conclusions
The results of the study confirmed the presence of ghrelin in non-functioning adenomas and somatotropinomas, also after farmacotherapy. Further studies would allow a better understanding of pituitary tumours pathogenesis.

Growth and development

P348

Secular height changes in Greek conscripts

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Introduction
In some developed countries, the acceleration of physical development has already reached a plateau.
Objective
Aim of our study was to examine whether an improvement of the body height of adult men is still observed in Greece and to correlate the body height with socio-economic and demographic factors.
Research methods and procedures
The height of 3068 Greek conscripts, aged 18–26 years, was measured and analyzed according to their socio-demographic characteristics, level of education (according to the years of obligatory education) and type of residence (urban or rural). Our data were compared with those of similar studies performed in 1968 and 1990.
Results
The mean height (±S.D.) of the recruit soldiers was 177.4 (±7.0) cm. Mean height according to the type of residence and educational level is shown in Table 1.

Table 1

Years of schooling		Urban	Rural	
		Mean height (±S.D.) cm		*P*
	Total	178.1 (7.1)	176.4 (6.7)	<0.0001
≤9	174.4 (7.0)	174.6 (7.8)	174.2 (6.3)	NS*
≥10	177.9 (6.9)	178.3 (6.9)	176.8 (6.7)	<0.0001
P	<0.0001a	<0.0001	<0.0001	

*Non significant.

From 1990 to 2007 mean height increased from 175.7 (S.E.M. 0.15) cm to 177.4 (S.E.M. 0.22) cm, corresponding to 1 cm per decade, $P<0.0001$. From 1968 to 1990 mean height increased from 167.9 to 175.7 cm, corresponding to 3.5 cm per decade.
Conclusions:
In the last 38 years a continuous significant increase of Greek men's final height was shown. However, in the last 16 years a deceleration of the rate of the improvement of the final stature was observed. The socio-economic status and the type of residence have a significant influence on body height. It appears that the male Greek population has still not exhausted its genetic potential.

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Population-based data of quality of life assessment of growth hormone deficiency in adults (QoL-AGHDA)

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Objective
Age- and gender-specific reference values for quality of life (QoL) measures are important to assess the impact of growth hormone deficiency (GHD). The objective of this study was to develop population-based data for the QoL-AGHDA instrument for Germany and to compare this data with corresponding Qol-AGHDA scores of the German KIMS cohort during growth hormone treatment, to investigate the association between the Qol-AGHDA score and the IGF-1 values in the German KIMS cohort as well as in the background population.
Design
For the purpose of this study, a questionnaire was developed that contained the QoL-AGHDA as well as questions recording an individual's general situation and social functioning. The questionnaire was sent out to a sample of 3,925 individuals drawn from a cross-sectional study in Germany. Corresponding data for 651 patients were retrieved from the German KIMS cohort. Moreover, the association between the Qol-AGHDA score and the IGF-1 values in the KIMS cohort as well as in the background population were analysed.
Results
The mean QoL-AGHDA scores for the general population were 4.5 (4.6) for men (women), respectively, compared with 8.8 (9.1) for KIMS patients before starting growth hormone replacement. For both males and females differences in mean QoL-AGHDA scores between general population and patients were statistically significant for all age categories ($P<0.05$). In the general population, the mean QoL-AGHDA score for each category of self-assessed health status increased progressively, indicating a poorer QoL as health status declined.
Conclusions
This study reports QoL-AGHDA scores for a population-based sample of Germany and confirms the extent of QoL impairment in patients with GHD in comparison with the general population.

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A study of IGF-1 serum concentrations and kinetics of growth hormone assays in stimulation tests used for diagnosing insufficient somatotrope secretion

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Introduction
The diagnosis of insufficient somatotrope secretion (growth hormone deficiency or GHD) is important in children.
In France, a child is considered to have a deficit if no serum GH concentrations greater than 20 mIU/l are recorded in 2 different stimulation tests. One of the tests must be 'coupled', i.e. combining 2 pharmacological agents.
However, this 20 mIU/l threshold does not take account of the type of test and large response variances that can be reported, depending on the product used. Nor does it factor in the patient's weight, age and pubertal development.
The goal of this study was to evaluate the interpretation of these tests vis-à-vis the legal threshold of 20 mIU/l depending on the product administered, and to compare it to IGF-1 results obtained, according to the standard reference values given by the supplier according to age.
Materials and methods
Chemiluminescent assays of hGH were performed in our laboratory in 2007 using Beckman Coulter's DXI instrument and somatomedin C assays were conducted using the Immulite 2000 from DPC (Siemens). HGH assays were calibrated to the international standard IS 98/574, as recommended.
Results
Of the 430 dynamic tests and regardless of the product(s) used, only 50% showed a positive response, with a secretion peak above 20 mIU/l. This percentage varied according to the product used:
- ornithine (51.3%), insulin (22.8%), glucagon (42.8%), L-Dopa (50%)
- clonidine–betaxolol (28.6%), glucagon–betaxolol (64.8%), glucagon–propanolol (69%), kerlone–glucagon (65.5%)

In light of these results and because the highest GH concentrations are observed at very different times from one patient to the next, it is important to comply with the different protocol times to avoid overlooking an unrecorded peak.
Despite the relationship between GH and IGF-1, the percentage of low IGF-1 concentrations in patients with a positive response to dynamic tests (15% of the 73 tests with ornithine) does not differ considerably from that observed in those presenting a response <20 mIU/l (19.7% of the 66 tests with ornithine). Therefore, IGF-1 assays cannot be used as a substitute for dynamic tests in diagnosing a GHD.
Keywords: hGH, IGF1, dynamic tests, GHD

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45,X/46,XX mosaicism: clinical implications in adulthood

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Introduction
Turner syndrome is well known, but prognosis for 45,X/46,XX mosaicism under 30% of aneuploidy has not been established. We realised a retrospective study among women aged 21–43 years, to evaluate differences in clinical features and biological parameters between patients who had sex chromosome mosaicism diagnosed incidentally and controls women.
Material and methods
Among infertile population, 71 women with sex chromosome mosaicism (45,X/46,XX) range from 4 to 28% were compared to 72 controls (46,XX). We evaluated clinical changes for menarche, menses, premature ovarian failure, height and body mass index. Assessments for early-follicular-phase blood values of FSH, LH, inhibin B, estradiol and TSH were measured at our laboratory. At last, we wondered if spontaneous fertility and pregnancy outcomes were influenced by such an aneuploidy.
Results
Eight percentage or more of aneuploidy was responsible for a lower height compared to controls (160 ± 6 vs 165 ± 6 cm; $P=0.01$). Sex chromosome aneuploidy was positively correlated to BMI ($P=0.0001$) and negatively correlated to menarche ($P=0.045$); moreover, menarche occured earlier when aneuploidy reached 10% or more (12.3 ± 1.0 vs 13.5 ± 1.3 years; $P=0.01$). There was no difference between the groups for FSH (8.41 ± 2.6 UI/l versus 8.28 ± 4.0 UI/l; $P=0.85$), LH, estradiol (50.6 ± 23.7 vs 51.7 ± 32.1 pg/ml; $P=0.81$), Inhibin B (55.5 ± 36.5 vs 57.4 ± 33.7 pg/ml; $P=0.80$) and TSH (1.74 ± 0.8 vs 1.83 ± 1.1 micUI/ml; $P=0.64$). Premature ovarian failure incidence did not differ between the two groups. Spontaneous abortions were more frequent in study group (2.0 ± 0.9 vs 1.2 ± 0.4; $P=0.01$) and recurrence was positively correlated to aneuploidy percentage ($P=0.008$).
Conclusion
Sex chromosome mosaicism is responsible for clinical changes since 8% of aneuploidy, corresponding to main phenotypic features of Turner syndrome.

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Evaluation of insulin sensitivity in growth hormone (GH) deficient children before and at the end of rhGH therapy

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Introduction
Therapy with recombinant GH (rhGH) allows GH-deficient children (GHD) to reach an adequate adult stature. Previous studies have reported a significant rise in serum insulin levels during GH therapy. It has been demonstrated that insulin resistance is a risk factor for the development of DMT2, atherosclerosis, dyslipidemia and hypertension.
Subjects and methods
Aim of our study was to evaluate changes in insulin sensitivity in a group of GHD children longitudinally followed during rhGH treatment. We measured fasting glucose and insulin levels and after oral glucose tolerance test (OGTT) in 11 GHD children (seven males and four females) at three times: 1) before starting of GH therapy (BT); 2) during the last year of therapy (T1); 3) 6 months (T6) after stopped therapy. At BT children presented age of 9.2 ± 0.7 years, height SDS -1.9 ± 0.2, while during T1 age was 15.4 ± 0.3 years, height SDS -1.2 ± 0.2. GH treatment was administered subcutaneously at a mean dosage of 0.2–0.3 mg/kg per week.
Results
No children showed impaired glucose tolerance or DMT2 during the therapy. Basal glucose levels were similar among the three times. Glucose levels in response to OGTT were statistically higher at T1 compared to BT and T6 ($P<0.007$). Fasting and during OGTT insulin levels were higher at T1 compared to BT and T6 ($P<0.03$). HOMA-IR index at T1 was higher compared to BT and T6 ($P<0.03$). Insulin sensitivity index as ISI was lower at T1 compared to BT and T6 ($P<0.01$).
Conclusions
Insulin sensitivity decreased during rhGH therapy, even if without the onset of impaired glucose tolerance, and was restored after stopping treatment. It remains to be clarified if this variation of insulin sensitivity during and after rhGH treatment could be related to changes in body composition and pubertal development or to rhGH treatment itself.

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Impaired Se metabolism provokes gender-specific growth defects in mice

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Background
Selenoproteins are involved in oxidative stress defence, cell signalling and hormone metabolism. Accordingly, impairment of selenium (Se) metabolism or transport results in a complex phenotype as exemplified in selenoprotein P knockout mice (SePP-KO). This mouse model is characterized by a disrupted metabolism of Se resulting in neurological and growth defects. Hypothesis: Se impairs regular tissue development by modifying growth signal biosynthesis, anabolic responses or oxidative stress levels within endocrine glands or target tissues.
Methods
Male and female wild-type, heterozygous and SePP-KO mice were raised on regular chow with defined Se-content. The expression of growth-relevant genes was studied in target tissues by qPCR and Northernblot analyses at 35 or 42 days of age. Serum markers like IGF-1 and IGF-BPs were determined by ELISA.
Results
Male SePP-KO mice displayed progressively reduced body weight gain starting two weeks after weaning. In contrast, female SePP-KO mice had a reduced bodyweight right from the beginning, but body mass differences diverged not further during the observational period. Significant differences in mRNA levels (57% reduction versus wild-type, $P<0.01$) and IGF1 serum levels (23% reduction versus wild-type, $P<0.05$) were exclusively observed in males. These findings underline the importance of Se for regular growth and health status and highlight medically important gender-differences in Se metabolism.
Conclusion
Se metabolism, Se status and transport are involved in tissue growth and body mass regulation. Our findings on the impact of gender on the SePP-KO phenotype may help to understand sex-specific effects of Se-supplementation in the clinics during disease prevention programs and therapeutical trials.
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Potential ageing effects in long-term cultured mouse neurospheres

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Neural cells are isolated from forebrain of 14.5 days old mouse embryos. In selective conditions these cells cluster forming sphere-like structures – neurospheres (NSCs). NSCs are heterogeneous structures containing only 2–5% of stem cells and progenitors being reportedly capable to transdifferentiate. Initially, aim of this study is to establish optimal culture conditions prior to investigation of transdifferentiation capacity of transplanted NSCs in kidney and adrenal glands. It was reported low reproducibility of such experiments but the age of transplanted NSCs was not considered as significant issue. In fact, it is generally assumed that progenitors and stem cells remain intact in long-term culture and therefore convenient for potential transplantation. In spite, we hypothesized that potential ageing effects of overall sphere certainly will impact the fraction of stem cells and progenitors. This assumption was tested exploring distinct aspects of ageing in long-term NSC culture. Potential alterations that

might occur due to ageing were monitored within 1–16 weeks of culturing. Initially, tremendous structural and numerous chromosomal aberrations were observed upon 16 weeks of culturing. This was accompanied with p53 up regulation. Moreover, p53 engagement may indicate higher apoptotic rate as self-defense mechanism in order to prevent further DNA burden. Telomere shortening was considered as senescence indication what may imply reduced number of cell divisions from certain time point. Telomere length measurement revealed decrease after 5 and 16 weeks. Although with jeopardized overall homeostasis, NSC unexpectedly displayed gradually elevated capacity to form spheres even in seeded low cell density up to 16 weeks culturing. Increased capacity to form spheres is accompanied with decreasing ability to differentiate into neural lineages. Genetic instability and diminished differentiation capacity seem to be a consequence of long term culturing implying potential transformation.

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Health-related quality of life in constitutionally tall children and adolescents

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Objective
To evaluate the health-related quality of life (HRQoL) in children and adolescents with constitutional tall stature (CTS).
Materials and methods
Sixty-two patients (20 males and 42 females) with CTS aged 9.3–16.4 years (12.9±2.4 years, mean±S.D.) were enrolled in this study. Sixty-eight normal subjects (24 males and 44 females) aged 9.8–16.2 years (13.1±2.1 years) served as controls. The evaluation of the HRQoL was performed using validated Russian version of 23-item PedsQL 4.0 (pediatric quality of life inventory) generic core scales.
Results
Mean total score (TS) and psychosocial health summary score (PHSS) were significantly lower in CTS females than in controls (mean TS: 69.4±3.4 vs 81.5±2.3; $P=0.02$; mean PHSS: 65.2±3.1 vs 80.8±2.7; $P=0.012$). Similar results were observed in parent proxy-reports (mean TS: 63.2±2.2 vs 81.3±2.1, $P=0.004$; mean PHSS: 60.4±3.3 vs 79.8±2.2; $P=0.006$). In contrast the physical functioning score was not significant different from that determined in controls. Also there were no significant differences in HRQoL parameters between CTS males and control group.
Conclusions
Females with CTS rated their HRQoL considerably lower than healthy controls, the domains of social and emotional functioning being particularly affected. The parents' ratings were considerably lower than those of the patients.

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Utility of 2-h nocturnal assessment of growth hormone (GH) secretion as a screening procedure in diagnosing GH deficiency in short children: own observations

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Background
Nocturnal profile of GH secretion is proposed to be a screening diagnostic procedure for GH deficiency (GHD) in short children. Every screening procedure must be characterised by very high sensitivity and good specificity. The data concerning the cut-off level between normal and subnormal nocturnal GH secretion are scarce and non-consistent.
The aim of the study was to assess the clinical utility of 2-h nocturnal profile of GH secretion in diagnosing GHD in children.
Material and methods
Nocturnal GH peak during 2-h assessment (5 samples every 30') was compared in the 2 groups of children, classified as: 1/ GHD – decreased both GH peak in 2 stimulating tests (<10 ng/ml) and IGF-I secretion (IGF-I SDS <−1.5 for age and sex ($n=24$), 2/ idiopathic short stature – normal both GH peak in stimulating tests and IGF-I secretion ($n=27$). ROC analysis was performed in order to assess the sensitivity and specificity of nocturnal GH peak for different cut-off levels.
Results
The best accuracy of 2-h nocturnal GH profile was found for the cut-off level of GH peak=16 ng/ml, with the sensitivity of 87.5% and specificity of 59.3%. The sensitivity on the level of 95% (acceptable for screening procedures) required increasing the cut-off value up to 20 ng/ml. Using the same cut-off level=10 ng/ml decreases the sensitivity of the examination to only 56.2%, with a slight increase of specificity – up to 74.1%.
Conclusions
Nocturnal 2-h profile of GH secretion may be a screening tool in diagnosing GHD, however with the cut-off level higher than for GH stimulating tests. There is no evidence for using the same cut-off values for GH peak in nocturnal profile as that established for stimulating tests.

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Abstract withdrawn

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Comparison between the results of 2-h and 6-h assessment of nocturnal growth hormone (GH) secretion in short children - preliminary report

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Background
Assessment of nocturnal GH secretion is useful tool in diagnosing GH deficiency (GHD) in children. The time period of the evaluation, the number of blood samples, as well as the choice of cut-off value for this examination – are still the matter for discussion.
Aim
The aim of the study was to compare the results of 2-h and 6-h assessment of nocturnal GH secretion in children with suspected neurosecretory dysfunction of GH secretion (NSD).
Material and methods
The analysis comprised the results of 2-h and 6-h assessment of nocturnal GH secretion in 33 short children with GH peak normal in stimulating tests (>10 ml) but decreased in 2-h nocturnal assessment (<16 ng/ml – i.e. the cut-off value with most accuracy in diagnosing GHD, according to ROC analysis) and decreased IGF-I secretion. The 6-h nocturnal profile was performed after approximately 6 months from the first evaluation. The blood samples for GH measurement were obtained in both tests every 30 min.
Results
GH peak in the first 2-h test was 8.5±4.5 ng/ml, being significantly ($P<0.05$) higher in the second test: 12.3±6.5 ng/ml during 2 h and 13.5±6.9 ng/ml during 6 h of profile. The results of the second evaluation were consistent with those of the first test in 24 children, while in the remaining 9 cases nocturnal GH secretion in repeated assessment presented over the cut-off level. In the 6-h profile, the highest GH peak was observed during first 2 h in 26 patients, while later – only in 7 children. However, in just 1 case only, the earliest value that exceeded the cut-off level was obtained later than during first 2 h of the test.
Conclusions
Repeated assessment of nocturnal GH secretion may prevent overdiagnosing NSD in children. It seems no necessary to assess 6-h nocturnal GH profile in such cases.

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Lipid peroxidation as possible mechanism of apoptosis activation in low birth weight children

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Children born with low birth weight (LBW, below 2500 g) exhibit slower development with deficit of height susceptibility to recurrent infections, especially of respiratory tract and, in adulthood, an increased risk of developing syndrome X. One possible explanation could be an enhanced elimination of cells by apoptosis.
The aim of this study was evaluate of mechanism of lipid peroxidation and activity of caspase 3 in children with low birth weight.
Subjects for study were 10 children with LBW and growth retardation (SDSHV < -1.8) and high frequency of apoptotic cells in cultures of lymphocytes and with the 50 kb domain on the DNA electrophoretic profiles, aged 4–11. Control group was 30 children with birth weight above 2700 g, aged 4–11.
Plasma total cholesterol, HDL- cholesterol, HDL_2-cholesterol, HDL_3-cholesterol, LDL- cholesterol, lipid peroxidase (LPO). Activity of caspase 3 was estimated in supernatant blood cells.
The study protocol was approved by Ethics Committee of Wroclaw Medical University.
Results
The levels of HDL_2-cholesterol, LPO and BCL2 were higher in LBW children than in the control group, so that activity of Caspase 3, the level of HDL_3-cholesterol was markedly lower, whereas total HDL and LDL concentrations did not differ between LBW and the control group.
In children with LBW the concentration of LPO negatively correlated with HDL_3 cholesterol ($r=-0.77$, $P<0.05$). Activity of Caspase 3 positively correlated with HDL_2-cholesterol concentration. ($r=0.91$, $P<0.05$). No significant correlations were found in the control group.
Conclusions
It is known that lipid peroxidation products activate apoptosis in human lymphocyte cultures through the direct stimulation of caspase 2 and caspase 3. Enhanced lipid peroxidation in blood of LBW children could be deduced from our data which showed the increase of LPO activity in those children, accompanied with the decrease of HDL_3-cholesterol level. Since HDL_3 is efficient in prevention of LDL non-enzymatic, Haber-Weiss peroxidation, we suggested that both enzymatic and non-enzymatic lipid peroxidation could be elevated in LBW children. These results were in accord with increased frequency of apoptosis, found in cultures of lymphocytes obtained from venous blood of LBW children.

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Study of dimer and oligomers of human growth hormone

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There is a high heterogeneity of human growth hormone (hGH), variation rising from the different genes in pituitary and placenta, alternative splicing, post-translational modifications, oligomerization and binding to the growth hormone binding protein (GHBP). Distinguishing between different variants remains problematic and there is still quite little information about the proportion of these variants in circulation of both healthy individuals and of patients with growth hormone disorders. Studying the abundance of dimer and oligomers in different physiological and pathological conditions is highly interesting since these forms of hGH will have longer half lives in circulation. In this experiment, 22 kDa hGH was expressed by HEK 293 cells and the hGH secreted in supernatant was studied. The western blot has shown, that in addition to monomer, hGH was also produced as dimer and oligomer. The dimer and oligomers are partially reduced by the reducing agent breaking down the disulfide bonds. Nevertheless, the dimer and oligomers can be detected even in reducing conditions, implicating that part of the dimer and oligomers are formed covalently otherwise than by disulfide bonding. A non-denaturing method based on fractionation with FPLC followed by high-sensitivity time-resolved immunofluorometric assays has turned out promising for the study of dimer and oligomer. The peaks measured by the immunoassays for monomeric versus dimeric and oligomeric hGH offered valuable information on the proportion of these forms. The function and structure of hGH dimer and oligomers as well as their relationship to hGH monomer need to be distinguished by further studies.

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Changes in cortisol and insulin during pregnancy in relation to basal metabolic rate (BMR)

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Cortisol rise during pregnancy is connected with fetal maturation. Together with the increase of insulin resistance it might have a role in allocation of nutrients between mother and fetus. Cortisol and insulin secretion during pregnancy seems to be induced by fluctuations in BMR. Here, we try to identify relationship between BMR and secretion of cortisol and insulin during pregnancy.
In 25 healthy women (age range 20–39 years; mean $\pm$ S.D., 28.6 ± 4.4 kg/m^2) body weight (BW), body mass index (BMI), BMR, basal insulin, and cortisol were measured in gestational weeks 12, 26 and 36. Homeostatic model index (R_{HOMA}) and individual differences (Δ) between values at 36 and 12 week of gestation for each variable were calculated. We used a ventilated hood system (Deltatrac Metabolic Monitor; Datex Instrument-arium Corp., Helsinki) to measure CO_2 and oxygen consumption during 20 min period. Insulin and cortisol were measured by RIA method. In statistical assessment repeated – measures analysis of variance and Pearson product moment were used.
Both BMI and BMR significantly rose with advancing of pregnancy ($P<0.001$ for both). Cortisol increased from 396.9 ± 173.2 nmol/l at gestational week 12, to 775.1 ± 354.1 nmol/l and 800.0 ± 285.0 nmol/l at gestational weeks 26 and 36, respectively ($P<0.001$, for both). Similarly, insulin secretion was augmented (from 7.9 ± 3.0 mIU/l to 9.1 ± 3.9 mIU/l and 11.7 ± 5.4 mIU/l; $P=0.001$). R_{HOMA} index rose, but this was less pronounced ($P=0.042$). We also found close positive correlation between ΔBMR with ΔBMI ($r=0.47$, $P=0.008$), Δinsulin ($r=0.40$, $P=0.038$) and Δcortisol ($r=-0.42$, $P=0.03$).
The rise of maternal serum cortisol and insulin suggest the activation of hypothalamo-pituitary–adrenal axis. Negative relationship between the increase of cortisol and BMR reflects cortisol induced decrease of fetal metabolic rate in late pregnancy. These may suggest brain involvement in control of energy homeostasis during pregnancy.

Growth factors

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Does IGF-BP3 assay contribute to the diagnosis of GHD in children?

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Background
Diagnosing GDH in children remains matter of debate due to variability in results obtained with pharmacological tests, one child may fail one test, but pass another. Spontaneous GH secretion assessment is costly, time consuming, and results may show poor correlations with those obtained with provocative tests. To add to diagnosic power of provocative tests, testing IGF-I and IGF-PB3 have been suggested. In recent years, however, the contribution of IGF-PB3 assay to diagnosis of GHD has been questioned ( ).
Objective
To evaluate the contribution of IGF-PB3 assay to the diagnosis of GHD.
Population and methods
Retrospective case study. Boys and girls aged 0–18 years who attended our Paediatric endocrinology clinic for short stature and/or follow-up post-irradiation, and had at least one provocation test for GH secretion assessment. We excluded those with hypothyroidism, Laron syndrome, severe malnutrition, chronic renal failure and liver failure.
Results
Fifty-eight children were enrolled and grouped according to results as GHD (+) (19 cases) and GDH (−) (39 cases). IGF-I and IGF-BP3 assay was carried out in 88% and 62% respectively, both groups were comparable for age, sex, BMI, target height, pubertal stage and bone age. There was a difference in maximum GH peak obtained between GDH (+) and GHD (−) groups (41.8 mUI/l ± 21.7 versus 11.5 ± 5.9 mUI/l, $P<0.00001$, respectively). No difference was found between groups with regards to IGF-I *Z*-scores and IGF-BP3 *Z*-scores. There was, however a positive correlation between IGF-I *Z*-scores and IGF-BP3 *Z*-scores ($r=0.50$; $P<0.0016$).

Conclusions
– IGF-BP3 assay contributes poorly to diagnosis of GHD, with 24% Sensitivity and 92% Specificity respectively.
– Due to lack reference values adjusted for age, sex, and pubertal stage, we do not recommend its routine use.

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Does administration of octreotide acetate prevent development of diabetic nephropathy? an ultrastructural evaluation of the effects of octreotide acetate on kidney tisssue in Streptozotocin induced diabetic rats

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Background and aims
Growth hormone and insulin-like growth factors to be involved in the pathogenesis of diabetic kidney disease. The aim of this study was ultrastructural evaluation of octreotide acetate on kidney tissue in streptozotocin (STZ) induced diabetic rats.
Material and methods
Twenty-four male Spraque–Dawley rats divided into 3 groups; control (C), diabetic (D) and diabetic with octreotide aceatate (DO). The all diabetic rats were treated with 4 IU/d human insulin. Octreotide acetate at the dose of 400 μg/kg per daily was applied intraperitoneally in DO groups rats. All rats were followed for a month and sacrified after cardiac blood samples were obtained. Both kidneys of all rats were obtained and weighted. All thin sections were stained with uranyl acetate and lead citrate and were examined by JEOL-TEM-1010 electron microscope and photo samples were obtained.
Results
Mean kidney weights of diabetic rats was higher than control rats (1.15 ± 0.12 and 1.68 ± 0.26 g respectively, $P < 0.001$). Mean kidney weights of diabetic rats plus octreotide was also higher than control rats (1.29 ± 0.11 g, $P < 0.02$). Ultrastructural findings of kidney were normal in control group. On the contrary, invagination in podocyte nucleus, dilation in golgi cystern and tubulus, obliteration in pedicel and capillary fenestrata were seen in diabetic (D) group. In octreotide (DO) group; a marked increment in mesangial matrix, dissociation among proximal tubulus cells and thickening in glomerular basement membrane were determined.
Conclusions
Experimental diabetes mellitus may lead to ultrastructural lesions in kidney tissue in even relatively early period of diabetes, and although there was little beneficial changes, a long acting somatostatin analoque, octreotide acetate, could not prevent development of diabetic nephropathy.

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Measurement of neutralising antibodies to human interferon-β by quantitative analysis of type 1 interferon inducible 6–16 mRNA

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Therapeutic interferon-β (IFN-β) preparations have been approved for treatment for multiple sclerosis (MS). However, the development of neutralising antibodies (NAbs) against IFN-β in MS patients reduces therapeutic efficacy. Early detection of NAbs is critical for the modification of treatment regimes. IFN-β exerts its biological effects by binding to receptors on target cells and stimulating the expression of IFN-β-inducible genes. Measurement of endogenous mRNAs for these genes can form the basis of functional bioassays. In this study we have used two approaches, quantitative reverse transcription-polymerase chain reaction (qRT-PCR) and branched DNA (bDNA) technology to develop efficient, sensitive and robust non-viral assays for the quantification of IFN-β NAbs in patients with MS. We show that the rapid (4 h) induction of the type I IFN-inducible 6–16 mRNA in A549 lung carcinoma cells is quantifiable by qRT-PCR and by bDNA assays. The induction of 6–16 mRNA is sensitively and reproducibly concentration-dependent for IFN-β stimulation and is comparable for real-time PCR and bDNA. In both assay systems, a sigmoidal dose response curve was obtained within the range 0.6–625 pg/ml IFN-β with a maximal response obtained between 156 and 625 pg/ml IFN-β. This is in broad agreement with data from antiviral assays in which maximum protection was obtained between 78 and 156 pg/ml IFN-β. Measurement of 6–16 mRNA by real-time RT-PCR and bDNA is readily adaptable for the detection and measurement of NAbs against IFN-β. Thus, sera from patients receiving IFN-β therapy (21 months) effectively neutralised IFN-β-stimulated 6–16 mRNA expression (100% neutralisation at a serum titre of 1:600; 50% neutralisation at a serum titre of approximately 1:4000), whilst pre-treatment serum from the same patients had no effect at the highest concentration tested. These data suggest mRNA-based assays could be used for bioactivity measurements of therapeutic products and also in a clinical setting for the monitoring of patients receiving IFNβ treatment.

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Association between serum levels of insulin-like growth factor-I and development of congestive heart failure: a prospective study in a normal population

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Background
The growth hormone system (growth hormone, GH and insulin-like-growth-factor I, IGF-I) might be implicated in congestive heart failure (CHF). In experimental models IGF-I increases cardiac contractility and reduces apoptosis of myocytes exposed to ischemic injury. In clinical studies GH-therapy has been used in CHF. One previous population based investigation showed that low levels of IGF-I was associated with an increased incidence of CHF. The result of this investigation was limited by lack of echocardiographic examination at baseline and the established riskmarker B-type natriuretic peptide (BNP) was not taken into consideration.
Objective
To examine the relationship between levels of IGF-I and the risk of development of CHF.
Method
A population based prospective study of 584 individuals (age 50–89 years) without a history of CHF and with normal systolic function at baseline assessed by echocardiography (left ventricular ejection fraction $\geq 50\%$). Development of CHF was ascertained after 5-years follow-up.
Cox proportional hazard regression analyses were used for the risk calculations. Results are given by P values and hazard ratio, HR (95% confidence limits) per 1 standard deviation increase in logarithmically transformed IGF-I level.
Results
Nineteen patients developed CHF (3.3%). They had significantly higher baseline levels of age-adjusted IGF-I levels compared to the rest of the population, 89 vs 72 ng/ml, $P = 0.01$ (IGF-I adjusted to a 70-year old individual).
Age-adjusted IGF-I was associated to increased risk of development of CHF, HR = 1.64 (1.15–2.35), $P = 0.007$. A multivariable model adjusted for age, levels of N-terminal proBNP (NT-proBNP), the presence of hypertension, atrial fibrillation and diabetes did not attenuate the association, HR = 1.67 (1.16–2.40), $P = 0.006$.
Conclusions
In a large middle-aged and elderly normal population high level of IGF-I was an independent riskfactor for development of CHF. The result was unexpected and in contrast to a previous reported protective role of IGF-I.

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Effects of growth hormone and of insulin-like growth factor-I on iron-induced lipid peroxidation in rat liver and porcine thyroid homogenates

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Bivalent iron (Fe^{2+}), which initiates the Fenton reaction ($Fe^{2+} + H_2O_2 + H^+ \rightarrow Fe^{3+} + {}^{\bullet}OH + H_2O$), is frequently used to experimentally induce oxidative damage to macromolecules. Growth hormone (GH) and the main mediator of its action – insulin-like growth factor-I (IGF-I) – are involved in oxidative processes, lipid peroxidation (LPO) included.

The aim of the study was to evaluate the effect of GH and/or IGF-I on iron-induced LPO in rat liver and in porcine thyroid homogenates. In order to determine the effect of GH and/or IGF-I on basal LPO, the homogenates were incubated in the presence of GH (100, 10, 1.0, 0.1, 0.01, 0.001, 0.0001 μg/ml) or IGF-I (1000, 100, 10, 1.0, 0.1, 0.01, 0.001, 0.0001 μg/ml) or GH (100 μg/ml) + IGF-I. In order to study their effects on iron-induced LPO, the homogenates were incubated with $FeSO_4$ (15 μM or 40 μM, for the liver and for the thyroid, respectively) + H_2O_2 (0.1 mM or 0.5 mM, for the liver and for the thyroid, respectively) and, additionally, with GH and/or IGF-I. The level of LPO was expressed as the amount of malondialdehyde + 4-hydroxyalkenals (MDA + 4-HDA). GH and/or IGF-I did not affect basal LPO in either tissue. In rat liver homogenates, GH did not affect iron-induced LPO in any way, whereas IGF-I (0.001, 0.0001 μg/ml) enhanced the process. In porcine thyroid homogenates, GH, in its lowest two concentrations, completely prevented, whereas in other used concentrations, it enhanced iron-induced LPO. In turn, IGF-I, in all the used concentrations, enhanced iron-induced LPO in the porcine thyroid. In conclusion, GH and/or IGF-I may directly contribute to oxidative balance in the liver and in the thyroid under physiological conditions but, in case of induced oxidative stress, they may reveal prooxidative effects, which fact does not support their application in the treatment of disorders associated with increased oxidative damage.

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Protective effects of growth hormone and of insulin-like growth factor-I against lipid peroxidation in iron sensitive rat tissue

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Iron participates in the Fenton reaction ($Fe^{2+} + H_2O_2 + H^+ \rightarrow Fe^{3+} + {}^{\bullet}OH + H_2O$), the most basic reaction of oxidative stress. This transition metal is required to keep oxidative balance, although iron overload is associated with enhanced oxidative damage and cancer initiation. Growth hormone (GH) and insulin-like growth factor-I (IGF-I) are also involved in oxidative processes, lipid peroxidation included.
The aim of the study was to evaluate the *in vivo* effect of GH, IGF-I and/or iron on lipid peroxidation in different rat tissues.
Male Wistar rats were administered iron as ferrous sulphate ($FeSO_4$; 3 mg/100 g b.w., on the 8th day of the experiment) and/or GH (0.2 IU/100 g b.w., once daily, for 8 days), and/or IGF-I (2 μg/100 g b.w., once daily, for 8 days). The level of lipid peroxidation products – malondialdehyde + 4-hydroxyalkenals (MDA + 4-HDA) – was measured spectrophotometrically in rat brain, lung, small intestine, liver, kidney, testis, spleen, and serum.
Among several examined tissues, only the small intestine and the brain appeared to be sensitive to oxidative effects of either GH, or IGF-I and/or iron.
Iron injection significantly increased lipid peroxidation only in the rat small intestine and that effect was completely prevented by either GH or IGF-I. In the rat brain, GH significantly decreased the basal lipid peroxidation, whereas IGF-I, either used alone or together with iron, significantly increased lipid peroxidation level.
In conclusion, not all rat tissues are equally sensitive to iron-induced lipid peroxidation, with only small intestine revealing such a property. GH and IGF-I possess some ability to prevent iron-induced oxidative damage in iron sensitive tissues, whereas contributing by themselves to oxidative imbalance in other tissues.

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Leptin and ghrelin serum levels during an oral glucose tolerance test in patients with growth hormone deficiency with and without growth hormone substitution

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In GH deficiency (GHD), fat mass is elevated compared to normal subjects. Leptin is positively related to fat mass, BMI and food intake, while ghrelin correlates negatively. During an oral glucose tolerance test (OGTT), leptin increases in obese subjects and generally does not change in normal weight subjects. Ghrelin decreases during OGTT. We performed a cross-sectional study in order to investigate the influence of GH-substitution on leptin and ghrelin in GHD.
Ghrelin and leptin were measured during 3 h OGTT in 52 GHD (21f/31m, median age 51.5 years (range 27–82)). Twenty-two GHD were on GH substitution (GH-sub) for a median of 10 years (2–42). Thirty patients were not substituted for at least 2 years (non-sub). Leptin was measured using an immunfluorometric in-house assay, total serum ghrelin by a radioimmunoassay (Phoenix Pharmaceuticals, USA). Body composition was measured by dual-energy-X-ray-absorptiometry.
Age and BMI (GH-sub 26 kg/m^2 (21–42) versus non-sub 29 kg/m^2 (22–66)) were not significantly different between GHD groups. Fat mass was significantly higher in non-sub (37% (20–52) vs 31% (13–54), $P < 0.01$), so were basal leptin (16 μg/l (3.3–89) vs 7.5 μg/l (0.5–130), $P < 0.05$) and the area-under-the-curve (AUC) of leptin ($P < 0.05$). Baseline ghrelin (GH-sub 145 ng/l (83–280) vs 113 ng/l (61–270)) and AUC of ghrelin were slightly but not significantly lower for non-sub compared to GH-sub. Leptin and ghrelin decreased significantly after glucose (maximum at 60 min) in both groups ($P < 0.001$). The lower fat mass of GH-sub caused by lipolytic GH might lead to lower leptin levels. The reason for decreasing leptin levels during OGTT in both groups remains unclear. Ghrelin levels were not significantly different between groups possibly due to the counterbalance between the suppressive effect of GH and the stimulating effect of reduced fat mass. GH has no effect on ghrelin regulation during OGTT.

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New occurrence of diabetes mellitus in patients with adult onset GH deficiency (GHD) on GH therapy is dependent on the presence of metabolic syndrome at baseline: data from the Hypopituitary Control and Complication Study (HypoCCS)

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Patients with adult onset GHD (AO-GHD) manifest features of the metabolic syndrome (MetS) (abdominal obesity, dyslipidemia and insulin resistance), a condition associated with increased risk of diabetes mellitus (DM).
We assessed metabolic status before and after 2 years of GH treatment and the occurrence of *de novo* DM in 712 patients with AO-GHD from HypoCCS drawn from the US (32.2%) and Europe (67.8%). Patients were divided into four BMI categories (<25, 25–<30, 30–<35, ≥35 kg/m^2), and MetS defined by the National Cholesterol Education Program (NCEP) criteria. *De novo* DM was recorded from reporting of a new event and/or newly initiated diabetes treatment.
The baseline prevalence of MetS was 43.0% with a linear increase across BMI categories from 11.0% to 73.9%. The baseline prevalence of DM was related to BMI and the presence or absence of pre-existing MetS, being 3.1 vs 18.8% for a BMI <25 kg/m^2 and 6.7 vs 43.5% for a BMI ≥35 kg/m^2, respectively. GH treatment did not change the prevalence of MetS (43.0 vs 44.2%). In those without pre-existing MetS ($n = 406$), DM developed in 0.0, 2.4, 0.0 and 0.0% for BMI categories <25, 25–<30, 30–<35 and ≥35, respectively. In those with MetS ($n = 306$), DM developed in 7.7, 11.9, 6.3 and 18.8% for the same BMI categories, respectively.
In summary, the prevalence of MetS is high in adults with GHD and is unchanged by 2 years of GH treatment. However, pre-existing MetS and BMI were strong determinants of the development of DM. Patients with AO-GHD with pre-existing MetS are at increased risk of developing DM during GH treatment, a risk that is worsened by increasing BMI. Greater vigilance should be exercised for early identification and therapy of DM in these patients.

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Associations of anthropometric parameters with serum IGF-1, TSH, prolactin and testosterone levels

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Background
Obesity is a major risk factor for the development of chronic diseases and a determinant of cardiovascular disease. Divergent associations between obesity and hormonal changes have been reported. The objective of the present study was to analyse the associations between different anthropometric measurements and serum hormone levels including insulin-like growth factor 1 (IGF-1), thyroid-stimulation hormone (TSH), prolactin, and testosterone.
Methods
A total of 2186 women and 2111 men aged 20–79 years from the population-based cross-sectional Study of Health in Pomerania (SHIP) were included in the analyses. Serum IGF-1, TSH, prolactin, and testosterone levels were determined by immunochemiluminescent procedures. Body height, weight and waist as well as hip circumferences were measured. Body mass index (BMI), waist-to-hip ratio (WHR), and waist to height ratio (WHtR) were calculated.
Results
Our analyses revealed negative associations between all considered anthropometric parameters and serum IGF-1 levels in women and men as well as serum testosterone levels in men. Furthermore, whereas in women anthropometric parameters except BMI and hip circumferences were positively associated to serum TSH and prolactin levels, no such associations were present in men.
Conclusion
Our results argue for interrelated hormone production and secretion associated with visceral fat. Furthermore, our analyses displayed that the use of visceral fat measurements like waist circumference, WHR and WHtR should be preferred for the assessment of associations between body fat and hormone levels.

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The cytosine-adenine (CA) repeat polymorphism in the promoter region of the insulin-like growth factor-1 (IGF-1) gene is not associated with the GH dose in GH-deficient adults

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Objective
A cytosine-adenine $(CA)_n$ microsatellite repeat polymorphism in the promoter region of the insulin-like growth factor-1 (IGF-1) gene has reported to be associated with IGF-1 serum levels, birth weight, body height, bone mineral density and risk for type 2 diabetes and myocardial infarction. Carriers and non-carriers of the most frequent allele (length 192 base pairs (bp)) showed significantly different total IGF-1 serum levels. This polymorphism may directly or indirectly influence growth hormone (GH)-mediated regulation of IGF-1 secretion.
Aim of this study was to test the hypothesis that the 192 bp polymorphism is associated with the GH dose of GH-deficient adult patients receiving recombinant GH-treatment.
Patients and methods
The 192 bp polymorphism was determined in 133 German adult patients (66 men, 67 women; mean age 45.4 years $\pm$ 13.1 S.D.; majority Caucasian) with GH-deficiency (GHD) of different origin, derived from the prospective KIMS Pharmacogenetics Study (approval was obtained from the local ethical committee). Patients received GH-treatment for 12 months with finished dose-titration of GH and standardized IGF-1 measurements. GH dose after one year of treatment, IGF- and IGF-SDS values and anthropometric data were analyzed by 192 bp polymorphism genotypes (homozygotes for the 192 bp allele; heterozygotes; non-carriers).
Results
The genotype distribution followed Hardy-Weinberg-equilibrium ($P=0.06$). Genotype groups showed no significant differences in the required GH dose after 1 year of GH-treatment ($P=0.61$), IGF-1 serum concentrations ($P=0.78$) and IGF-1 SDS ($P=0.93$) after adjusting for the confounding variables gender, age and body mass index.
Conclusion
The 192 bp polymorphism was not associated with the responsiveness to exogenous GH in GHD adults. As effects of non-GH factors on the IGF-1 levels (insulin-levels, sex steroids, nutrition, liver function) in GH-deficient patients were minimized, GH-driven regulation of IGF-1 levels seems not to be influenced by the 192 bp polymorphism in these patients.

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Acromegaly is associated with high plasma fibrinogen and C reactive protein values after normalization of growth hormone and IGF-1

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Background
Acromegaly is associated with increased morbidity and mortality from cardiovascular disease. Several studies indicate that reduction of growth hormone (GH) to <1 μg/l or normalization of serum IGF-I reduces mortality to expected levels. Inflammatory markers, such as C-reactive protein (CRP) or haemostatic markers, such as fibrinogen have emerged as important cardiovascular risk markers in the general population.
Objective and design
The objective of the present study was to assess serum levels of high sensitive CRP and fibrinogen in patients with normalized GH and IGF-1 values after treatment compared to the background population. This case-control study included 32 patients (18 women) with acromegaly after successful treatment and 128 controls of a population-based study matched by sex and age.
Results
We detected a higher body mass index (30.3 vs 27.2 kg/m^2; $P=0.02$) and more cases of diabetes (23 vs 9%; $P=0.05$) in patients with acromegaly compared to controls. However, there was no differences for hypertension, smoking and lipids between both groups. Patients revealed normalized but even higher mean IGF-1 values than controls (129.5 vs 232.7 ng/ml; $P<0.05$). Moreover, patients with acromegaly showed higher values of high sensitive CRP and fibrinogen. After adjustment for confounders, patients with acromegaly had higher odds of serum CRP and fibrinogen values compared to controls. In a linear regression model, elevated fibrinogen values were positive associated with IGF-1 values and acromegaly.
Conclusion
Normalized but higher IGF-1 values after treatment of acromegaly are associated with increased high sensitive CRP or fibrinogen values. This might be a possible explanation for the revealed increased all-cause mortality in acromegalic patients compared to the general population even after treatment.

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Low insulin-like growth factor (IGF) I, IGF binding protein-3 and acid labile subunit (ALS) levels in young ischemic stroke: a prospective case-control study

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Animal and observational studies suggest that the IGF axis is involved in the pathogenesis of ischemic stroke; moreover, endogenous IGF-I could influence the evolution of ischemic stroke in humans and thereby contribute to an improved clinical and functional outcome. To assess and correlate the IGF-I axis with biochemical parameters in young patients experiencing ischemic stroke (32 aged 15–45 years,19F, BMI: 25.5 $\pm$ 3.4 kg/m^2), we evaluated IGF-I, IGFBP-3, ALS, homocysteine, antithrombin, anticardiolipin antibodies (ACA), lupus anticoagulans (LAC), Protein S and C, fibrinogen levels and lipid profile 6–12 months after stroke. The results were compared with those of 32 age, BMI and sex matched controls. IGF-I levels were lower in patients than in controls (86.3 $\pm$ 76.6 vs 262.9 $\pm$ 50.5 μg/l; $P<0.001$) and lower than 2 SD in 24 patients (75%). IGFBP-3 and ALS levels were also significantly reduced in patients than in controls (3394.8 $\pm$ 801 vs 3782 $\pm$ 762.8 ng/ml, $P<0.05$; 10.2 $\pm$ 3.8 vs 21.2 $\pm$ 27.5 μg/ml, $P=0.000$; respectively. Conversely as expected, homocysteine, total cholesterol and fibrinogen levels were higher in patients than in controls ($P<0.001$). Significant difference was found between patients and controls in Protein S ($P<0.001$) and C ($P<0.05$), while no difference was found in ACA and LAC levels between two groups. In addition in the patients' group, IGF-I levels were significantly correlated with homocysteine levels ($r=0.411$, $P<0.05$).
In conclusion a significant impairment of IGF-I and ALS secretion was found in young patients 6–12 months after ischemic stroke. Whether this represents a causal or casual finding remain to be clarified.

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Pituitary hormone axes in constitutionally tall adolescents

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Objective
To evaluate the pituitary hormone axes in adolescents with constitutional tall stature (CTS).
Materials and methods
About 120 patients (54 males and 66 females) with CTS aged 9.6–16.5 years (13.6±2.7 years, mean±S.D.) were enrolled in this study. Sixty-eight normal subjects (24 males and 44 females) aged 9.8–16.2 years (13.1±2.1 years), who were comparable in socioeconomic and nutritional terms, served as controls. Growth hormone, insulin-like growth factor-I (IGF-I), IGF-binding protein-3 (IGFBP-3), luteinizing hormone, follicle-stimulating hormone and prolactin levels were measured.
Results
In comparison with controls, CTS children showed a slightly lower concentration of IGFBP-3 and a higher concentration of IGF-I but these differences were not significant. In contrast the IGFBP-3/IGF-I molar ratio was significantly lower (0.7±0.1 vs 1.1±0.2; $P<0.001$) in CTS children than in controls. The concentrations of other pituitary hormone in CTS-affected subjects were not significant different from those determined in controls.
Conclusions
This study has demonstrated that in CTS the most components of pituitary hormone axes are normal, but the IGFBP-3/IGF-I molar ratio is increased, and, therefore, a greater availability of free IGF-I for target tissues may be responsible for overgrowth in CTS.

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TIMP-1 regulates the secretion of soluble VEGF receptor 1 in human endothelial cells

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Objectives
Vascular endothelial growth factor (VEGF) and its soluble receptor (sVEGFR-1) are key regulators in human ovarian angiogenesis. VEGF promotes blood vessel growth while sVEGFR-1 is a VEGF antagonist. A promising candidate in sVEGFR-1 regulation is TIMP-1 (tissue inhibitor of metalloproteinases 1), which is involved in extracellular matrix remodelling. The aim of our research is to determine if TIMP-1 influences sVEGFR-1 secretion in endothelial cells.
Methods
Pooled human endothelial cells were seeded in 24-multiwell plates. Twenty-four hours afterwards, TIMP-1 was downregulated in the cells by RNA interference using four TIMP-1 siRNAs (small interfering RNAs) and two unspecific siRNAs. After four days incubation, TIMP-1 and sVEGFR-1 concentrations were quantified in culture supernatant by enzyme-linked immunosorbent assay (ELISA). Control endothelial cells were used without additional treatment.
Results
As shown in ELISA assays, TIMP-1 concentration in the endothelial culture supernatant was significantly reduced between 65% and 78% by using the four TIMP-1 siRNAs when compared to untreated control cells. According to this, sVEGFR-1 concentration was also significantly decreased between 39% and 69% in the cell culture supernatant by using the specific siRNAs. Endothelial cells transfected with the unspecific siRNAs, however, showed similar TIMP-1 and sVEGFR-1 amounts like untreated controls.
Conclusion
The amount of soluble VEGF receptor 1 was significantly reduced in the TIMP-1 knockdown experiment. Therefore, sVEGFR-1 secretion in endothelial cells can be regulated by TIMP-1.

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Overexpression of IGFBP-2 in transgenic mice affects muscle protein accretion, skeletal myofibre growth and metabolism

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To elucidate the functional role of IGFBP-2 for *in vivo* skeletal muscle growth and function, skeletal muscle cellularity and metabolism as well as body growth and composition have been studied in a transgenic mouse model overexpressing IGFBP-2. Postnatal growth rate of transgenic mice ($n=20$) was clearly reduced from day 21 of age by 6–8% ($P<0.05$) compared with non-transgenic controls ($n=37$). At the final day of live (day 72 of age) body protein and moisture were lower, whereas fat percentage was higher in the IGFBP-2 transgenes ($P<0.05$). The weights of the lower leg and of the *M. rectus femoris* (RF) were reduced by 14% ($P<0.0001$), which was associated with higher DNA ($P<0.05$) at unchanged RNA and protein concentrations in RF. The reduction in muscle mass resulted mainly from slower growth of myofibres in size as seen from smaller cross sectional areas (by 12%; $P<0.01$), but not from decreases in the number of fibres. Analyses of metabolic fibre type composition and enzyme activities revealed that muscle metabolism was shifted to the glycolytic pathway of energy generation. The proportion of white (glycolytic) fibres was increased at the expense of intermediate (oxidative/glycolytic) fibres and, likewise, the activity of lactate dehydrogenase (LDH) was elevated ($P<0.01$). All differences observed between transgenic and non-transgenic mice were more pronounced in males as derived from significant genotype by sex interactions. The results suggest that IGBP-2 inhibits postnatal skeletal muscle growth and modifies muscle metabolism.

Neuroendocrinology

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Turner syndrome: body composition and psychological state

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Introduction
Turner syndrome (TS) is female genetic disorder arrising from loss of X-chromosome material, associated with characteristic physical neuropsychological features.
Objective
Examine peculiarities of body composition, cognitive functions, emotional state, quality of life (QoL) of TS patients after discontinued growth hormone (GH) therapy.
Patients and methods
Eighteen girls (age 21.47±4.09 years) with diagnosis of TS established in childhood. GH therapy for at least five years (discontinued immediately to the beginning of the study).
Height, weight, waist-to-hip ratio, body composition, cognitive functions (trail making test, TMT), emotional state (profile of mood state, POMS), QoL (special questionnaire for growth disturbances) in TS patients were compared to that of controls.
Results
Height (148.8±5.7 vs 168.4±5.1 cm, $P<0.001$), weight (50.4±7.9 vs 61.3±8.6 kg, $P<0.001$) in TS girls were significantly lower than in controls, but differences in body mass index were not significant. Lean body mass (36.31±4.02 vs 47.02±4.47 kg, $P<0.001$), water body mass (27.37±1.73 vs 32.39±2.52 kg, $P<0.001$) in TS patients were significantly lower than in controls. Waste-to-hip ratio in TS girls was significantly higher (0.82±0.06 vs 0.74±0.04, $P<0.001$) than in controls.
In emotional state tension-anxiety (10.6±7.2 vs 6.7±3.9, $P=0.02$), depression-dejection (14.7±11.9 vs 8.5±6.3, $P=0.02$) were significantly higher, vigour-activity (13.1±4.8 vs 16.1±4.4, $P=0.04$) significantly lower in TS patients than in controls. TMT score (96.9±44.6 vs 58.1±15.4, $P=0.001$) was significantly higher (worse psychomotoric speed) in research group than in controls. QoL (9.5±5.2 vs 6.3±4.2, $P=0.03$) in TS girls was significantly worse than in healthy.
Conclusion
In conclusion, TS patients after discontinued GH therapy has lower height, weight, higher waist-to-hip ratio, lower lean body mass and water body mass, impaired cognitive functioning, altered emotional state, lower quality of life in comparison to normal girls of the same age.

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Can early evening melatonin treatment prevent relapse after sleep deprivation?

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Patients with winter depression (seasonal affective disorder) respond beneficially to sleep deprivation and bright light, but the mechanisms of these responses remain unknown. The study was designed to test whether afternoon/evening melatonin can prevent further relapse after sleep deprivation (presumably due to a pharmacologically induced advance shift of circadian phase). Compared to phase advancing by alteration of sleep-wake schedule or by bright light exposure, the melatonin intake is a more tolerated treatment procedure, and it provides a possibility of blind comparison between chronotherapeutic and placebo treatments. The depression was scored in 16 female patients with winter depression and 17 age-matched female controls before and after total night sleep deprivation and after subsequent 6-day administration of melatonin (0.5 mg) or placebo under double blind conditions. The melatonin intake was scheduled at 17:00 in order to produce a phase advance of circadian rhythms. Sleep deprivation resulted in 38% reduction of depression score in patients, but it did not reduce depression score in controls. After the subsequent treatment with placebo or melatonin slight but significant improvement of mood was found in controls. These treatments also stabilized the antidepressant response to sleep deprivation in patients. However, neither differential effect of melatonin and placebo on depression score nor alteration of habitual sleep timing was found in patients and controls. We conclude that the study results do not provide evidence for antidepressant potential of melatonin in patients with winter depression under realistic clinical conditions. The finding of stabilization of mood in patients with placebo suggests the contribution of psychological factors to therapeutic action of this and other innovative treatments for winter depression. The study was supported by the Russian fund of the Society for Light Treatment and Biological Rhythms.

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Reduction of food intake by insulin detemir in comparison to regular human insulin

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Systemic insulin is considered to serve as a major negative feedback signal in the central nervous regulation of food intake. Accordingly, euglycemic infusion of regular human insulin (RI) has been shown to reduce hunger in a dose dependent fashion. Due to its increased lipophility, the long-acting insulin analogue insulin detemir (DI) might cross the blood–brain barrier faster and in higher quantities than RI and exceed stronger anorexigenic effects. To test this hypothesis, we measured food intake after DI and RI administration. 15 healthy, normal-weight men were examined in two hyperinsulinemic euglycemic clamp experiments taking place in the morning after an overnight fast. In both conditions, an insulin bolus injection (DI, 90 mU/kg body weight; RI, 17.75 mU/kg) was followed by a steady 90 min insulin infusion (2.0 vs 1.0 mU/kg per min). To compensate for the relatively slower onset of the action of DI, a higher dosage was chosen to induce comparable peripheral effects of both compounds. Mean blood glucose levels during the clamp period were likewise comparable between conditions ($P<0.384$). Twenty minutes after infusion, subjects were allowed to eat *ad libitum* from a standardized breakfast buffet. ID reduced total food intake in comparison to RI (1257 ± 82 vs 1560 ± 139 kcal, $P<0.04$). This effect was also observed for the intake of protein ($P=0.004$), tended to be observed for the intake of carbohydrates ($P=0.063$) but not of fat ($P=0.201$). Although systemic contributions to the observed effects cannot be ruled out, our data suggest that compared to regular human insulin, insulin detemir induces stronger effects on brain networks that control food intake. They further support the notion of insulin as an anorexigenic feedback signal in the central nervous regulation of energy homeostasis.

P380

Pituitary insufficiency after traumatic brain injury and the coherence with psychological disorders

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Objective
In order to explain physical, cognitive and psychological disorders after traumatic brain injury (TBI), several studies have implicated a major role of posttraumatic pituitary insufficiency (prevalence 25–70%). Aim of this study was to determine the correlation between cerebral tissue damage, corresponding initial findings on radiographic imaging and pituitary insufficiency versus psychological disorders (like fatigue, nervousness, depression or excitability).
Material and methods
About 171 patients with TBI had been contacted, 55 (32%) agreed to participate in the study. Hormonal stimulation tests had been performed, either if the basal hormone screening revealed an abnormality or if the patient answered 'yes' in at least one question of the pituitary questionnaire.
The following data had been included into a multivariate analysis: initial GCS, GOS, pituitary questionnaire, age, prevalence of pituitary insufficiency and initial findings on radiographic imaging.
Results
Overall, 14 out of 55 patients (25.4%) presented with pituitary insufficiency; one of them with two hormonal deficits. Neuropsychological changes e.g. nervousness, excitability and depressive episodes were present in 67% of the patients.
Conclusion
About 67% of the patients developed neuropsychological disorders after TBI, whereas only 25% of the patients revealed any type of pituitary dysfunction. This might lead to the assumption, that general brain tissue damage has more influence, regarding the development of psychological disorders after TBI, than pituitary dysfunction.

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Review of pituitary adenomas diagnosed in Burgos (Spain)

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Introduction
Pituitary adenomas are the most common cause of sellar masses from the third decade on, accounting for up to 10 percent of all intracraneal neoplasms.
Objective
To review pituitary adenomas diagnosed in Burgos.
Patients and methods
It was a retrospective study in which we reviewed a total of 90 pituitary adenomas detected in Burgos from 1983 to our days.
Results
About 65% were macroadenomas and 35% were microadenomas. At the moment of diagnosis the median size of macroadenomas was 2.9 ± 1.9 cm and of microadenomas 0.5 ± 0.3. Macroadenomas had similar frequency in both sexes (30 cases in men and 29 cases in women) while microadenomas were diagnosed more frequently in women (26 vs 5 in men). The most frequent type was the non-functioning adenoma (42 cases), followed by lactotroph adenoma (20), corticotroph adenoma (12), somatotroph adenoma (11), thyrotropin-secreting adenoma (4) and gonadotroph adenoma (1). Corticotroph, lactotroph and tyrotropin-secreting adenomas were detected more frequently in women. The youngest at the moment of detection were the patients with corticotroph adenomas (35 ± 18 years). The oldest ones were the non-functioning adenomas (57.7 ± 16 years). The biggest ones were the non-functioning adenoma (2.6 ± 2.4 cm), followed by lactotroph (2.5 ± 1.5), somatotroph (2.3 ± 0.8) and thyrotrophin secreting adenomas (2 ± 0).
Conclusions
In our study we have a great number of macroadenomas and lactotroph adenomas are not the most frequent adenoma's type. It could be due to the way we have selected our patients. We have used the information that was recorded in our hospital's data base. These patients are the ones that were treated with surgery or radiotherapy, so many patients with lactotroph microadenomas have been lost. Three of our four thyrotropin-secreting adenomas died from hemorrhage complications. This association has not been described before.

P382

Residual adverse vascular risk and oxidative stress in treated adult panhypopituitarism

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Background
Adult hypopituitarism is associated with premature vascular mortality for which the underlying mechanisms are unknown but untreated GH deficiency is proposed as a potential contributor.
Objectives
To characterise vascular risk, including paraoxonase-1 (PON1; an antioxidant enzyme which preserves LDL against oxidation), in adults with treated panhypopituitarism.
Study subjects
The study had full ethics approval. Data are presented as mean ± S.D. or median (quartiles). Twenty-one panhypopituitary adults (group 1, aged 45 ± 15 years), on stable pituitary replacement (>9 months), including GH and 43 controls of similar age (group 2, age 49 ± 11 years) were studied.
Results
IGF-I SDS pre-GH replacement was −4.9 (−7.3, −2.95) and on stable GH replacement was 0.2 (−0.68, 1.03) in group 1 (normal range −2 to +2). Nine patients (5 female, age 52 ± 15 years) from group 1 had prolactin levels persistently below 50 mU/l. Median total daily glucocorticoid replacement was hydrocortisone 20 mg or equivalent. Triglycerides were higher in group 1 compared with group 2 (2.02 (0.98–5.1) vs 1.09 (0.48–5.7) mmol/l; $P<0.001$, as were CRP levels (3.0 (1.7–6.8) vs 1.1 (0.1–11.0) mg/l; $P<0.005$). HDL levels were lower in group 1 (1.17 ± 0.28 vs 1.51 ± 0.43 mmol/l; $P<0.005$) as was PON1 activity (60 (27–278) vs 129 (27–438) nmol/ml per min; $P<0.005$). Total cholesterol, calculated LDL, ApoB, ApoB/A1, Lp(a), adiponectin were similar between groups. BMI (32 ± 6 vs 25 ± 4 kg/m^2; $P<0.001$) and insulin levels (26.5 (1–123.7) vs 15.9 (5.6–25.8) mU/ml; $P<0.05$) were significantly higher in group 1. Within group 1, insulin levels were lower in the prolactin deficient subgroup (21.3 ± 19.9 vs 53.1 ± 38.1 mU/ml, $P=0.03$) but prolactin levels correlated strongly with BMI ($r=0.78$; $P<0.005$) only in prolactin non-deficient subjects.
Conclusions
Treated adult hypopituitarism is associated with residual adverse vascular risk during stable optimised conventional pituitary replacement, including GH. The observations may relate to the presence of associated obesity. Lower PON1 activity in panhypopituitarism is of interest because reduced PON1 activity is associated with oxidative stress and increased cardiovascular risk.

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Is total and acylated ghrelin secretion after oral glucose modified by acromegaly?

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Introduction
Although involved in feeding and weight homeostasis, stimulation of pituitary GH secretion is the best established action of stomach-produced hormone ghrelin. However, its role in regulation of GH secretion is not yet clear. Some evidence indicates that circulating concentrations of GH and/or IGF-I could influence ghrelin levels. The pathophysiology of ghrelin secretion in acromegaly (specially aftar an oral glucose tolerance test (OGTT) is unclear.
Objectives
To study circulating fasting acylated and total ghrelin levels, and their response to an OGTT in active acromegalic patients and normal control subjects matched for age, sex, and BMI, and their relation with glucose, insulin, and GH.
Patients and methods
We included nine patients with active acromegaly, and nine age, BMI and percentage body fat matched healthy subjects as controls. Patients with any degree of hypopituitarism were under appropriate and stable hormone replacement therapy. We obtained blood samples for glucose, insulin, GH, total ghrelin and acylated ghrelin at times 0, 30, 60, 90 and 120 min after 75 g of oral glucose.
Results
Fasting GH and IGF-I were statistically different between patients and controls: GH (μg/l) 6.7 ± 1.4 vs 0.8 ± 0.4, $P<0.01$; IGF-I (ng/ml) 414 ± 75 vs 86 ± 6, $P<0.01$. Fasting total ghrelin (pg/ml) was similar in patients and control group, 916 ± 132 vs 844 ± 169, P=NS. Fasting acylated ghrelin levels (pg/ml) were also similar in both groups, 65 ± 13 vs 74 ± 14 (Figure 1). In both groups, total ghrelin levels decreased during OGTT, and nadir total ghrelin was lower than fasting total ghrelin: patients 916 ± 132 vs 747 ± 95, $P<0.05$; controls 844 ± 169 vs 625 ± 90, $P<0.05$ (Figure 2). Also, in both groups, acylated ghrelin levels decreased during OGTT, with nadir being lower than basal levels: patients 65 ± 13 vs 42 ± 6, $P<0.05$; controls 74 ± 14 vs 37 ± 4, $P<0.05$ (Figure 3). We have found a negative correlation between fasting ghrelin (both total and acylated) and insulin levels (both fasting and post OGTT).
Conclusions
Our data suggest that circulating total and acylated ghrelin in acromegaly is regulated by insulin and not by GH hypersecretion.

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Hypothalamic–pituitary insufficiency following infectious diseases of the central nervous system

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Hypothalamic–pituitary insufficiency may have diverse causes. Infectious diseases of the central nervous system (CNS) may also affect the hypothalamus and/or the pituitary, although this has not been reported very often and not yet been studied systematically. It was the aim of this study to determine the incidence of hypothalamic–pituitary insufficiency in patients with previous infectious diseases of the CNS of different etiology.
Patient series. Basal and stimulated (insulin tolerance test) pituitary function testing was performed in 19 patients (13 males, 6 females, 38.7 ± 11.7 years) with previous neuroborreliosis, encephalitis or meningitis of mild to moderate clinical course following an interval of 26.1 ± 13.1 months after the acute event.
Four patients (21%, two males, two females) showed an isolated corticotrophic insufficiency (peak cortisol <181.25 μg/l). In patients reporting self-experienced fatigue (including all patients with a corticotrophic insufficiency) peak cortisol concentrations were lower than in patients not reporting fatigue (165.4 ± 38.9 vs 213.6 ± 18.4 μg/l; $P=0.002$). Two patients (11%, two males) showed an isolated borderline gonadotrophic insufficiency (basal testosterone between 2.4 and 3.0 μg/l). No patient had a somatotrophic or thyrotrophic insufficiency or diabetes insipidus, all had prolactin concentrations within the reference range.
Conclusions
Hypothalamic-pituitary dysfunction may develop in a relevant proportion of patients after infectious diseases of the CNS. Especially self-reported persisting fatigue might be suggestive for the presence of a corticotrophic insufficiency. The clinical picture of some patients might be misinterpreted as an ordinary postencephalitic syndrome. Further prospective studies investigating patients after infectious diseases of the CNS and the effects of hormone replacement therapy are warranted.

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The choice of therapy in acromegaly: results of treatment at a tertiary care hospital

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Objective
The aim of our study was to investigate the characteristics of the acromegalic patients followed at a tertiary Universitary Hospital and to evaluate the results of the recommended treatment protocols.
Patients and methods
All our acromegalic patients were included ($n=48$; 27 women). Demographic, hormonal, visual and imaging data at diagnosis and during follow-up, as well as treatments applied, were recorded.
Results
In 73.0% of the patients acromegaly was due to a pituitary macroadenoma. Of those under periodic surveillance, 68.2% were operated (60% of them in our hospital, 40% in other hospitals) and 36.4% were submited to radiotherapy. At the time of the study 88.6% of the patients were receiving medical therapy, 28.2% of

them as first-line treatment. Following actual criteria (Melmed *et al*. 2005), only one patient was cured by surgery. Considering age and sex-matched normal concentrations of IGF-I as a criteria of control, surgery resulted in disease control in 10% of the operated patients, while medical treatment controlled the disease in 76.9% ($P<0.05$). This percentage was 75.0% in the group of patients who received medical therapy as adjuvant of surgery and/or radiotherapy and 81.8% in the group of patients who received medical treatment as first-line treatment ($P=$NS). After surgery visual function improved in 6 patients, hypopituitarism improved in 6, and some degree of *de novo* hypopituitarism developed in 5. Results of the surgical procedures performed in our centre were similar to the results obtained in other hospitals. Of those who received medical therapy as first-line treatment, the tumor size decreased in 45.5% and in the rest no significant changes were observed during follow-up.
Conclusions
Not all centres obtain the results reported in the literature in terms of disease control and morbidity after surgical treatment of growth hormone secreting tumors. It is possible that in some hospitals first-line medical treatment should be chosen, unless the patient has visual disturbances, as long as it is not clear that partial surgical removal of the tumor significantly improves response to medical therapy or that it reduces its costs.

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Isolated GH deficiency of adult-onset in the KIMS database: prevalence, clinical presentation, and response to GH replacement

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Of the ~12 500 GH treated subjects with adult-onset GH deficiency (GHD) in KIMS (Pfizer International Metabolic Database), 3744 with multiple pituitary hormone deficiencies (MPHD) and 367 (9%) with isolated GHD (iGHD) were eligible for baseline analysis. Subjects met the following inclusion criteria: 1) never received GH prior to entry in KIMS, 2) had stimulation tests with insulin or glucagon (GH <3 μg/l), arginine (GH <0.4 μg/l), or arginine + GHRH (cut-offs based on BMI), 3) had organic disease as the cause of hypopituitarism. About 60–65% of subjects in both groups had pituitary adenomas. At baseline iGHD subjects were younger than MPHD subjects (46.7 vs 48.7 years, $P<0.01$), had a shorter history of GHD (1.4 vs 2.1 years, $P<0.001$), a lower lean body mass (53.6 vs 56.3 kg, $P<0.05$) but similar BMI, a higher IGF-I SDS (-1.2 vs -1.7, $P<0.001$), and a worse QoL-AGHDA score (12.8 vs 11.6, $P<0.05$). Longitudinal analysis after 2-years of GH replacement compared to baseline showed a similar response in the iGHD ($n=180$) group compared to the MPHD ($n=2140$) group, the only difference being a more pronounced increase in IGF-I SDS (2.2 vs 1.7, $P<0.001$). Significant changes in the iGHD group were observed: waist (94.0 vs 95.2 cm, $P<0.05$; LDL cholesterol (3.4 vs 3.7 mmol/l, $P<0.001$); triglycerides (1.8 vs 2.0 mmol/l, $P<0.02$); QoL-AGHDA score (improving from 13.0 to 8.4, $P<0.001$); IGF-I SDS (0.5 vs -1.2, $P<0.001$).
In conclusion: iGHD patients should receive GH replacement since they respond as well as MPHD patients.

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Efficacy of slow release formulation of lanreotide (Somatuline Autogel® 120 mg) in patients with acromegaly: single center experience

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Somatuline Autogel® 120 mg (SATG, Ipsen, Sweeden) is a high dose sustained release aqueous gel formulation, supplied in a prefiled syringe and given by deep subcutaneous injection. The aim of this cross-sectional hospital based study, approved by the local ethical committee, was to investigate efficacy and tolerability of SATG given every 4 weeks in patients with active acromegaly.
Twenty patients (9f, 11m) aged 42.9 ± 17.8 (range 17–68 years) with active acromegaly were treated with lanreotide in our center for median of 15 months (average 13.3 ± 9.2 months; range 3–30 months). Eighteen patients were operated, one was irradiated after the operation and two patients were primarily treated with lanreotide. Eight out of twenty patients (40%) were replaced for hypopituitarism. Four patients with mixed somatotrophe-lactotrophe adenomas and hyperprolactinemia were additionally treated with dopamine agonists.
In 75% of patients (15/20) IGF-1 levels ($x\pm$S.D.) decreased significantly during treatment with lanreotide from 591.6 ± 294.4 to 346.7 ± 160.8 ng/ml, $P=0.0002$. In 35% (7/20) of patients normalization of IGF-1 levels was achieved and in another 30% (6/20) regression of the pituitary tumor rest was evident on the control MRI scans. Four patients (20%) achieved both normalization of IGF-1 levels and regression of the tumor rest. One patient achieved shrinkage of the tumor rest with no decrease in IGF-1 level. Four patients (20%) did not respond to treatment with lanreotide.
As for tolerability, one patient discontinued treatment with lanreotide after first injection due to chest discomfort and hypotensive reaction and was excluded from the study. Others tolerated lanreotide well.
In conclusion Somatuline Autogel 120 mg is an effective and well tolerated therapy for acromegaly.

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Smoking causes increased androgen levels in perimenopausal women

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Introduction
Several studies have investigated determinants for androgen levels in men, but few studies have included women. Epidemiological evidence has suggested that cigarette smoking has an anti-oestrogenic effect in women, but the effects of smoking on steroid hormone metabolism are not fully understood. We investigated whether there is a relationship between androgen levels and smoking in perimenopausal women.
Material and methods
We examined 75 perimenopausal women, 30 were smokers and 45 were non-smokers. All women were between the ages 40 and 55 and presented with a history of menstrual cycle irregularity of at least 6 months duration but not longer than 1 year of amenorrhea. We measured plasma testosterone (T), dehydroepiandrosterone sulphate (DHEAs), follicle stimulating hormone (FSH), luteinizing hormone (LH) and estradiol (E2) levels in these perimenopausal women. Pearson's correlations were applied to evaluate the relationship between plasma hormone levels and smoking.
Results
The smokers had a higher level of testosterone and DHEAs than the non-smokers ($P<0.01$). Serum FSH, LH and E2 were similar in the two groups.
Conclusions
Our results demonstrate that perimenopausal women who smoke have a higher serum concentration of testosterone and DHEAs than non-smokers. These results confirm previous reports that cigarette smoking does not affect serum estradiol in perimenopausal women and also support previous findings of increased levels of some adrenal steroids in postmenopausal women smokers.

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Body fat excess and GH-stimulated levels in adult patients with Prader–Willi syndrome

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The GH response to standard provocative tests is significantly lower in adult patients with Prader–Willi syndrome (PWS) than obese controls with similar body mass index (BMI). Nevertheless, BMI is an inadequate measure of body composition in PWS, because PWS harbour a higher fat mass than simple obesity, under the same degree of weight excess. This study evaluated either the GH response to combined GHRH + arginine administration and the fat body mass, by dual energy X-ray absorptiometry, in a group of 11 PWS adults (8 females, aged 20.1–41.1 years), in comparison to those obtained in a group of 10 patients with

essential obesity (8 females, aged 23.5–45.8 years), matched for BMI and percentage of total body fat (FM%). The study protocol was approved by the local Ethical Committee. Statistical analysis was performed by *t*-test for unpaired data, and using analysis of variance for parametric and nonparametric (Mann–Whitney test) data, where appropriate. Mean (+S.E.M.) FM% was similar in PWS and obese subjects (53.0+1.5 vs 51.5+1.0, respectively). The GH response to GHRH+arginine was significantly lower in PWS patients (GH peak 5.4+1.3 μg/l; area under the curve (AUC) 311.2+72.5 μg/l per h) than obese subjects (GH peak 21.0+4.1 μg/l, $P<0.005$; AUC 1241.1+272.8 μg/l per h; $P<0.01$). In PWS group, the GHRH+arginine induced GH rise in patients with del15q11–q13 was significantly higher than those observed in subjects with UPD15 (GH peak 7.7+1.7 μg/l vs 2.7+1.0 μg/l, $P<0.05$; AUC 458.5+91.0 μg/l per h vs 134.4+46.0 μg/l per h, $P<0.02$). These findings suggested that GH secretory pattern is different in PWS adults when compared to patients with simple obesity and that reduced GH secretion is not exclusively related to adiposity in PWS subjects. In addition, our data seem to indicate that GH secretory pattern is significantly different in PWS individuals having separate genetic subtypes.

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Cardiovascular risk factors in adult onset growth hormone deficiency (AO-GHD) without growth hormone replacement: follow-up period seven years

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Patients with hypopitutarism have higher cardiovascular morbidity and mortality rates than general population, as manifested by elevated fasting lipids and insulin resistance. The aim of our study was to evaluate the lipid levels and insulin responses during oral glucose tolerance test (OGTT) in adult onset growth hormone deficiency (AO-GHD), without growth hormone replacement during a follow-up period of seven years. Ten hypopituitary patients with operated non-functioning pituitary adenoma were investigated (5 female/5 male, mean age: 42.5±4.2 years; duration of hypopitutarism 14.3±2.5 years). Mean BMI at the beginning of the study was 27.9±1.7 kg/m^2, while after seven years-follow-up period was 28.6±1.1 kg/m^2, $P>0.05$). At baseline, cholesterol, triglyceride and HbA1c levels were measured and OGTT was performed. Mean peak, delta and areas under the curve (AUC) of glucose and insulin levels during OGTT were analyzed. The mean cholesterol level (6.1±0.5 mmol/l) and mean triglyceride level (1.8±0.3 mmol/l) did not change after seven years of follow-up (6.7±0.6 mmol/l and 1.8±0.3 mmol/l, respectively, $P>0.05$). The mean delta glucose level (4.1±0.6 mmol/l) and mean AUC glucose level (775±65 mmol/l per 120 min) were significantly higher after seven years of follow-up compared with these values at baseline (2.3±0.5 mmol/l and 605±54 mmol/l per 120 min, respectively, $P<0.05$). The HbA1c level was significantly higher after seven years of follow-up compared with baseline level (5.8±0.1 vs 5.1±0.1%, $P<0.05$). There were no differences in insulin responses during OGTT during the follow-up period, compared with baseline (mean delta insulin, 59.2±26.6 vs 70.2±18.5 mU/l, $P>0.05$; mean AUC insulin, 4763±1610 vs 5965±1271 mU/l per 120 min, $P>0.05$). In conclusion, AO-GHD have significantly higher HbA1c levels and glucose response during OGTT after seven years of follow-up, possibly due to ageing.

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Nocturnal HPA axis activity is blunted by increased plasma glucose concentrations

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Background
Secretory activity of the hypothalamo-pituitary–adrenal (HPA) axis typically increases during the second half of nocturnal sleep. Assuming that this rise in ACTH and cortisol levels occurs in response to the negative energy balance induced by nocturnal fasting and concomitant increases in cerebral glucose consumption during REM sleep, we examined the effects of glucose infusion on nocturnal HPA axis activity during wake and sleep periods.
Methods and findings
According to a 2×2 design, healthy men were infused with glucose (4.5 mg/kg×min) and saline, respectively, during sleep ($n=9$) or total sleep deprivation ($n=11$). Circulating concentrations of ACTH, cortisol, glucose, insulin, and leptin were measured and food consumption from a breakfast buffet presented on the subsequent morning was assessed. Independent of sleep, nocturnal secretion of ACTH and cortisol was suppressed by glucose infusion (glucose vs saline: ACTH, 11.93±0.77 vs 13.60±1.08 pg/ml; cortisol, 5.21±0.92 vs 7.20±0.79 μg/dl; each $P<0.05$). In the sleep group, glucose infusion enhanced REM sleep while reducing the time spent in sleep stage 2 (each $P<0.05$). Sleep deprivation per se was associated with a reduction in leptin levels compared to sleep ($P<0.05$). Following nocturnal glucose infusion, food intake was reduced in comparison to placebo in the wake but not in the sleep group ($P<0.05$ and $P>0.82$, respectively).
Conclusions
Our findings indicate that nocturnal HPA axis activity is blunted by increased plasma glucose concentrations, suggesting that the brain regulates nocturnal ACTH and cortisol release in response to the level of energy available in the form of blood glucose. Sleep does not appear to be critically involved in this glucose/glucocorticoid feedback loop.

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Macroprolactinemia seems to have a positive effect on platelet activation through ADP stimulation

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Objective
Platelet activation is a recently recognized characteristic of prolactin, which functions through the potentiation of ADP-induced P-selectin expression on platelets. Studies in hyperprolactinemic patients demonstrated atherosclerotic disorders related to insulin resistance; convenient milieus for platelet activation. To investigate the association between hyperprolactinemia and platelet activation related to P-selectin expression, we studied on hyperprolactinemic and normoprolactinemic patients.
Method
After exclusion of any factor that might interfere with platelet functions, 32 naïve hyperprolactinemic and 33 age-body mass index-matched normoprolactinemic, non-smoking premenopausal women were included; 30.6±8 vs 29.8±7.7 years, 26.8±5.4 vs 24.8±5.2 kg/m^2, prolactin 1889.8±886 vs 335.9±117.9 mU/lt. Measurements regarding insulin sensitivity; waist circumference, blood pressure, fasting plasma glucose, insulin and lipids were also matched. The flow-cytometry method was used to determine the ADP stimulated P-selectin expression of the platelets. Serum prolactin was measured before and after polyethylene glycol precipitation (PEG) in hyperprolactinemic group. The diagnosis of macroprolactinemia was regarded as certain if the prolactin recovery in a serum was <40%.
Results
The ADP stimulated P-selectin expression of the platelets was higher in hyperprolactinemic group; 14.2±15.5% vs 6.7±5.2%, ($P=0.01$). The frequency of macroprolactinemia was found to be 29%. There was a significant correlation between prolactin levels and ADP stimulated P-selectin expression before PEG ($r=0.3$, $P<0.02$). The ADP stimulated P-selectin expression rates were similar between macroprolactin negative (true hyperprolactinemia) ($n=21$) and macroprolactin positive ($n=11$) subgroups; 13.6±16.4% vs 15.3±14.4% ($P=0.7$). It kept being higher than the controls in both subgroups; 13.6±16.4%, ($P=0.03$) and 15.3±14.4%, ($P=0.005$), respectively.
Conclusion
Platelet activation is involved in the pathogenesis of atherosclerotic disorders related to insulin resistance. In this study, performed on completely matched group of cases regarding insulin sensitivity markers, hyperprolactinemia itself has been detected to bring an increased risk for platelet activation. It has also been clearly demonstrated that macroprolactinemia may cause platelet aggregation just as true hyperprolactinemia.

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A study of the serotoninergic tone in healthy subjects and diabetic patients treated by a clinical-paedagogic approach (Group Care) or traditional care

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Background and aims
Alterations of mood and depression may be associated with type 2 diabetes (T2DM) and impaired glucose metabolism in non-diabetic people. Central serotoninergic activity may modulate glucose tolerance via neuroendocrine effectors. The serotoninergic tone was assessed by measuring the hormonal response to a selective serotonin reuptake inhibitor, citalopram, in non-diabetic subjects and patients with T2DM followed by either a traditional approach or a clinico-paedagogic intervention (Group Care) proven to improve quality of life and reduce anxiety.
Materials and methods
Ten healthy subjects (age 53.20±3.92; males=5) and 13 patients with T2DM (age 60.92±3.52, known duration 17.38±5.85, males=10), 7 followed by Group Care and 6 by traditional care, underwent infusions of either citalopram 20 mg or saline over 120 min, in random order, at 8.30–9.00 am after overnight fasting. Samples were taken from an antecubital vein every 15 min for 4 h to measure circulating glucose, insulin, ACTH, free cortisol, DHEA, growth hormone (GH) and prolactin. Hormonal responses were calculated as differences between the areas under the curves after saline and citalopram (Δ-AUC).
Results
Citalopram increased ACTH and cortisol more than saline in healthy subjects ($P<0.026$ and $P<0.011$, respectively) and diabetic patients treated by Group Care ($P<0.056$ and $P<0.038$) but not in those followed by traditional care. The responses of DHEA, GH, prolactin, insulin and glucose to citalopram did not differ significantly from those to saline in any of the 3 groups. However, in the healthy subjects, basal glucose levels correlated directly with the GH Δ-AUC ($r=0.820$; $P<0.004$) and inversely with the insulin Δ-AUC ($r=-0.822$; $P<0.003$). The correlation between basal glucose and GH Δ-AUC was preserved in diabetic patients ($r=0.637$; $P<0.026$), in whom HbA1c correlated with basal glucose ($r=0.657$; $P<0.015$) and the insulin Δ-AUC ($r=0.864$; $P<0.012$), and the glycaemic Δ-AUC correlated with that of cortisol ($r=0.698$; $P<0.012$).
Conclusions
These results suggest that serotoninergic tone may modulate glucose metabolism through opposite effects on ACTH, cortisol, insulin and GH secretion. Diabetes may blunt the response of the pituitary-adrenal axis, though not in patients followed by a long-term intervention model that encompasses clinical as well as cognitive and emotional factors.

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Growth hormone (GH) treatment on atherosclerosis: results of a 5 years open, prospective, controlled study in male patients with severe GH deficiency

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Background
Severe GH deficiency (GHD) is associated with, increased cardiovascular (CV) risk and intima-media thickness (IMT) at major arteries.
Objective
To investigate the long-term (5-years) effects of GH replacement on insulin resistance (IR) syndrome (IRS) (at least two of: triglycerides levels ≥1.7 mmol/l, HDL-cholesterol levels ≤1.0 mmol/l, blood pressure above 130/85 mmHg, fasting glucose 6.1–7 or 2 h after glucose 7.7–11.1 mmol/l), and common carotid IMT.
Design
Interventional, open, prospective, controlled.
Patients
About 35 men with severe GHD aged <50 years (standard replacement therapy plus GH was given to 22 (Group A) and except GH in 13 (Group B)) and 35 healthy men age-matched with the patients as control.
Results
At baseline, 18 patients (51.4%) and two controls (5.7%; $P<0.0001$) had IRS. The patients with IRS had a longer estimated duration of GHD ($P=0.006$), lower IGF-I SDS ($P=0.002$) and higher IMT ($P=0.041$) than those without IRS. Mean IMT was significantly higher in the patients with ($P<0.001$) and without IRS ($P=0.004$) than in controls. During the study period, the use of lipid lowering drugs (92.3%, vs 13.6% and 34.3%, $P<0.0001$), glucose lowering drugs (69.2% vs 31.4% and 22.7%; $P=0.016$) and of anti-hypertensive drugs (61.5% vs 20.0% and 4.5%; $P<0.0001$) was higher in Group B patients than in controls and in Group A patients. After 5 years, IGF-I levels were normal in all Group A patients and remained lower than −1 SDS in 10 of 13 Group B patients. IMT at right and left common carotids significantly decreased only in Group A, while it significantly increased in controls and non significantly in group B patients. The prevalence of IRS significantly reduced only in group A patients, non significantly reduced also in group B patients while slightly increased in controls.
Conclusions
Severely GHD men have more frequently IRS and increased IMT at common carotid arteries than sex- and age-matched controls. Only in GH replaced GHD patients after 5 years was observed decreased IMT and decreased prevalence of IRS.

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Dopamine receptor expression and dopamine agonist effectiveness in corticotroph pituitary tumors: comparison with clinical, biochemical radiological and pathological features of patients with Cushing's disease

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Dopamine receptors (DR) are expressed in the majority of corticotroph pituitary tumors and the dopamine agonist cabergoline is effective in controlling cortisol hypersecretion in around 50% of patients with Cushing's disease (CD). In order to characterize the tumors expressing D_2 receptors and the profile of patients which might respond to the treatment with D_2 agonists, the current study has the aim to correlate D_2 receptor expression and the effects of cabergoline with clinical, biochemical and radiological features of patients, as well as to pathological features of tumors removed form the patients. The study included 72 patients with CD. In all patients, D_2 receptor expression, evaluated by immunohistochemistry (IHC), was correlated with the pathological features of the tumors and the characteristics of the patients, whereas in a subgroup of them, D_2 receptor expression, evaluated by RT-PCR, or the cabergoline responsiveness was correlated with the pathological features of the tumors and the characteristics of the patients. The results of the study demonstrated that D_2 receptor expression is significantly associated with the presence of neural pituitary tissue close to the tumors and/or nerve fibers within or surrounding the tumors, presumably expression of the tumor origin from the intermediate zone of the pituitary gland, as well as with the presence of a corticotroph hyperplasia rather than adenoma at the histological evaluation, and PRL staining within the tumor. Moreover, dividing the tumors according to the intensity of D_2 staining, those with the highest D_2 expression, are also associated with lower age, longer disease duration, a relative resistance to dexametasone and CRH and higher prevalence of hyperprolactinemia, together with an undetectable tumor at the imaging techniques and failure at neurosurgery. The initial response to cabergoline treatment was also associated with some of these characteristics of patients and tumors. However, the long-term responsiveness to cabergoline treatment was only associated with the tumor expression of the short isoform of D_2 receptor and/or D_4 receptors. In conclusion, the current study demonstrated that the corticotroph tumors expressing D_2 receptors derive from either the intermediate zone or the anterior lobe of the pituitary gland. Moreover, whereas the tumors originating from the intermediate zone generally express higher number of D_2 receptors and are associated to the best initial responsiveness to cabergoline treatment, those with the expression of short isoform of D_2, deriving from both the intermediate zone and the anterior lobe of the pituitary gland are associated with a long-term responsiveness to cabergoline treatment.

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The effects of growth hormone deficiency following moderate and severe traumatic brain injury on cognitive functions

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Hypopituitarism, in particular growth hormone (GH) deficiency, is common among survivors of traumatic brain injury (TBI). We investigated neurobehavioral consequences of TBI-induced GH deficiency (GHD) in 61 patients (aged 37.7 ± 1.7 years, 44 male/17 female) at least one year (mean, 3.9 ± 0.6 years) after moderate and severe TBI (mean GCS score 10.6). All patients were tested with standard neuropsychological battery (MMSE, TMT, RAVLT, RCF, BNT and WCST for executive cognitive functions). Serum samples for IGF-I, T4, testosterone (in males), prolactin and cortisol were taken at baseline and the GH/IGF-1 axis was evaluated by GHRH + GHRP-6 test. Three TBI patients with multiple pituitary hormone deficiencies were adequately replaced (except for GH). According to the established peak GH cut-off for normality >20 mcg/l and cut-off for severe GHD <10 mcg/l, TBI patients were divided in two groups: severely GHD ($n=9$, mean GH peak: 5.4 ± 1.0 mcg/l) and those with normal GH secretory capacity (GHS, $n=52$, mean GH peak: 34.4 ± 2.5 mcg/l). GHD TBI patients performed on WCST with significantly lower number of achieved WCST categories compared to GHS TBI patients (2.9 ± 0.5 vs 5.1 ± 1.0, $P<0.05$). Furthermore, GHD TBI patients have performed with more perseverative responses compared to GHS TBI patients (38.1 ± 2.1 vs 31.6 ± 3.9, $P<0.05$). In other tested cognitive variables there were no significant differences between GHD and GHS TBI patients. A significant inverse correlation was observed between GH peak response to the GHRH + GHRP-6 test and number of perseverative responses on WCST ($P=0.05$). In conclusion, GHD is a frequent consequence of TBI and can substantially influence executive cognitive functions, as demonstrated by neuropsychological testing.

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Pharmacological prevention of long-term neuroendocrine and behavioral changes in prenatally stressed rats

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Maternal stress modifies fetal programming of neuroendocrine development and behavior. These effects are mediated by maternal and fetal corticosteroids and opioids. We evaluated an ability of pharmacological prevention of long-term behavioral and hypothalamic–pituitary–adrenal (HPA) disorders in adult rat offspring induced by daily 1 h maternal immobilization during the last gestational week. Dexamethasone (Dex; 0.1 mg/kg per day) or naltrexone (Ntx; 10 mg/kg per day) treatments prior to pregnant rats stressing prevented impairment of copulatory behavior (increase of mounting latency, decrease of ejaculatory rates etc.) in adult male offspring. Both Dex and Ntx exerted protective effect with regard to diminishing adrenocortical and hypothalamic nordarenaline responses to an acute stress as well as decrease of stress-induced activation of $GABA_A$ and $GABA_B$ receptors tested with muscimol and baclofen in those animals. In prenatally stressed female rats, Ntx prevented enhancement of adrenocortical response to an acute sress while no protective effect of Dex has been found. One of the long-term consequences of prenatal stress was an impairment of adrenocortical response to noradrenaline infusion into the third brain ventricle: it was significantly diminished in females and increased in males. These changes were not observed both in males and females treated prenatally with Dex or Ntx. We concluded that Dex blockade of endocrine response to stress or Ntx blockade of opioid receptors can prevent pathological changes in sexual behavior and HPA noradrenergic and stress reactivity in prenatally stressed rats in gender-related manner.

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Genetic and clinical analyses in an Italian series of idiopathic hypogonadotropic hypogonadism

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Idiopathic hypogonadotropic hypogonadism (IHH) is a rare and heterogeneous disease due to defects of GnRH secretion or action. IHH could be associated or not with anosmia respectively identifying the Kallmann's syndrome (KS) or the normosmic IHH (nIHH). So far numerous causative genetic defects have been described, but very recent molecular genetic studies and animal models have opened novel perspectives. We are studying a series of 16 KS (14M,2F) and 18 nIHH (14M,4F). All patients have normal karyotype, low/normal gonadotropins in the presence of low sex-steroid levels and a blunted LH/FSH response after GnRH injection. *FGFR1, Ebf2, PROK2, PROKR2* genes were examined in all cases whereas *GnRHR* and *GPR54* gene analysis was limited to nIHH. The coding sequences of *GnRHR*, *Ebf2* and *GPR54* showed no abnormalities. Two novel mutations of PROKR2 (V158I, V334M) and one of PROK2 (G62D) gene were found in 1 nIHH female, 1 KS male patients and 1 nIHH male patient, respectively. The analysis of FGFR1 gene showed results of particular interest. The mutation D200E was found in two unrelated patients discordant for osmic function and bimanual synkynesia. The mutation F210X was instead found in 1 nIHH male patient that did not undergo puberty. This patient had a brother also affected with nIHH and absent pubertal development that was found to carry the same mutation. This mutation was therefore inherited from one of the parents who had had a normal puberty and was still unaffected at an age beyond 30 years. These variations are present in the heterozygous state in the patients according to the reported mechanism of haplo-insufficiency. The variable neurological phenotype (osmic function and bimanual synkynesia) associated with the same FGFR1 mutation and the highly variable IHH penetrance in our family represents additional arguments in favour of a multiple genetic origin of these defects.

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Four years of growth hormone (GH) therapy improves markers of cardiovascular risk in GH deficient (GHD) survivors of childhood acute lymphoblastic leukaemia (ALL)

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Survivors of childhood (CO) ALL treated with prophylactic cranial radiotherapy (CRT) often exhibit (GHD) and increased cardiovascular risk. The aim of this study was to evaluate the effect of 4 years of GH therapy on cardiovascular risk factors in 16 former ALL patients and to compare them with matched population controls after 4 years.

Sixteen former CO ALL patients (women = 8), aged 24–37 years, treated with CRT (18–24 Gy) and chemotherapy with confirmed GHD and controls were studied at baseline. After 4 years of GH therapy (median 0.5 mg/day) the investigations were repeated in the patients and in the control subjects.

Four years of GH treatment increased the serum IGF-1 significantly into the mid-normal range (SDS IGF-1 0.3) in the former ALL patients ($P=0.03$). Compared to baseline, a significant decrease in the plasma levels of glucose ($P=0.03$), apoprotein B (apo B) ($P=0.03$) and the ratio apo B/apoprotein A1 (apo A1) ($P=0.006$). Also HDL cholesterol ($P=0.03$) and apo A1 ($P=0.05$) increased significantly compared to baseline levels. Comparison between patients and controls after 4 years showed no significant difference. There was no difference in fatmass at baseline between patients and controls and four years of GH treatment did not change fatmass in the patients. The blood pressure showed no significant difference in patients after four years of GH.

In conclusion, 4 years of GH therapy to former CO ALL patients resulted in significant improvements in the plasma levels of glucose, apo B, apo A1, apoB/apoA1 and HDL, indicating a positive effect of GH on cardiovascular risk.

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Impaired microvascular reactivity and endothelial function in patients with Cushing's syndrome

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It is well known that hypercortisolism is associated with increased morbidity and mortality caused predominantly by vascular and atherosclerotic complications. The effect of hypercortisolism on microvasculature is less known. The aim of this study was to evaluate skin microvascular reactivity (MVR) in relation to arterial hypertension, diabetes mellitus and other possible influencing factors (fibrinolysis, oxidative stress and endothelial function) in patients with Cushing's syndrome (CS).

Twenty-nine patients with active CS, 10 patients at least 6 months after successful operation and a group of 16 control subjects were studied. Skin MVR was measured by laser Doppler flowmetry during post-occlusive (PORH) and thermal hyperemia (TH). Selected parameters of fibrinolysis, oxidative stress and endothelial activity were determined as well.

We found an impaired microvascular reactivity in patients with CS. Maximal perfusion during PORH and TH was significantly lower in patients with arterial hypertension ($P<0.01$, $P<0.05$, respectively) as was the velocity of perfusion increase during PORH and TH ($P<0.05$). The most expressed impairment of microvascular reactivity was present in patients with combination of arterial hypertension and diabetes mellitus. Impaired fibrinolysis, increased oxidative stress and endothelial dysfunction were present in patients with hypercortisolemia, however, increased concentration of ICAM-1 was found also in patients after operation as compared to controls ($P<0.05$). It documents the presence of persistent endothelial dysfunction also in cured patients.

Based on the obtained results, we conclude that the impairment of microvascular reactivity in hypercortisolism can be modulated by many factors but the most important is the presence of arterial hypertension. The combination of arterial hypertension and diabetes mellitus led to the most decreased microvascular reactivity in patients with Cushing's syndrome.

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Serum and glucocorticoid-induced kinase 1 mediates basal and stimulated POMC transcription in pituitary corticotrophs

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Cushing's tumours are ACTH-producing neoplasms of the pituitary gland. Their ACTH-oversecretion is autonomous and does not respond to the canonical inhibition of the hence also elevated cortisol. Current research in identifying new drug targets is mainly focused on designing therapeuticals that directly inhibit ACTH over-secretion from the adenoma. Serum and glucocorticoid-induced kinase 1 (Sgk1) is an early target gene of glucocorticoids in all cells studied. It is a PKB/Akt-like kinase that acts downstream of the phosphoinositol-3-kinase (PI3K) signal transduction pathway. PI3K is related to increased cell proliferation and hormone secretion in corticotrophs, and a target of recent drugs that reduce ACTH oversecretion.

We present here that Sgk1 is expressed in pituitary corticotrophs and localised in the perinuclear cytosol, as demonstrated by immunohistochemistry. Quantitative RT-PCR reveals that stimulation of the murine corticotroph tumour cell line AtT-20 with dexamethasone induces a rapid increase of Sgk1 transcription, accompanied by a reduction of the transcription of pro-opiomelanocortin (POMC). In parallel, dexamethasone induces a translocation of Sgk1 to the nucleus, as shown by Western blotting. Transient over-expression of Sgk1 in AtT-20 cells is associated with reduced POMC transcription. Moreover, siRNA-mediated down-regulation of Sgk1 causes an increase in POMC mRNA levels.

In summary, Sgk1 is a downstream target of dexamethasone in corticotroph cells. It controls basal and stimulated POMC expression and is a potential drug target for the treatment of Cushing's disease. Ongoing investigations are aiming to fully characterise the role of Sgk1 in corticotroph cells.

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Effect of hyperprolactinemia in male patients consulting for sexual dysfunction

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Objectives

The physiological role of prolactin in male sexual function has not been completely clarified. Aim of this study is the assessment of clinical features and of conditions associated with hyperprolactinemia in male patients consulting for sexual dysfunction.

Design and methods

A consecutive series of 2146 (mean age 52.2±12.8 years) male patients with sexual dysfunction was studied. Several hormonal and biochemical parameters were studied along with validated structured interviews (ANDROTEST and SIEDY). Mild hyperprolactinemia (MHPRL; PRL levels 420–735 mU/l or 20–35 ng/ml) and severe hyperprolactinemia (SHPRL, PRL levels >735 mU/l, 35 ng/ml) were considered.

Results

MHPRL and SHPRL were found in 69 (3.3%) and in 32 (1.5%) patients respectively. Mean age and the prevalence of gynecomastia were similar in the two groups and in subjects with normal prolactin values. MHPRL was not confirmed in almost one half of the patients, after repetitive venous sampling. Hyperprolactinemia was associated with the current use of antidepressants, antipsychotic drugs and benzamides. SHPRL was also associated with hypoactive sexual desire (HSD), elevated TSH, and hypogonadism. The association between HSD and SHPRL was confirmed after adjustment for testosterone, TSH levels and use of psychotropic drugs (HR=8.60 (3.85–19.23); $P<0.0001$). In a 6 -months follow up of patients with SHPRL testosterone levels and sexual desire were significantly improved by the treatment.

Conclusions

Our data indicate that SHPRL, but not MHPRL, is a relevant determinant of HSD. Gynecomastia does not help in recognising hyperprolactinemic subjects, while the use of psychotropic medications and HSD are possible markers of disease. In case of MHPRL, repetitive venous sampling is strongly encouraged.

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Effects of 6 months growth hormone treatment in GH deficient patients (GHD) long after traumatic brain injury (TBI)

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We studied 84 patients with neuropsychological disabilities at least 1 year after TBI. 24 patients had severe GHD (29%). The aim of this study was to evaluate the effects of six months GH treatment on quality of life and metabolism in this group.

GH treatment was initiated in 17 patients (71%), planned with 3, declined by one, contra-indicated in 2, 1 patient moved. It lasted more 6 months in 16 of them (14 men, mean age 36±12, mean delay after TBI 75±4, >36 months for 15 patients). GHD was isolated ($n=6$) or associated with ACTH deficiency ($n=9$) and/or TSH deficiency ($n=3$). GH treatment was initiated after the susbstitution of other deficiencies, at 0.3 mg/day. Dosage was increased in 25%, decreased in 25%. We suspected a defect of observance in 4 patients.The mean dosage at 6 months was 0.3 mg/d (0.2-0.4). IGF1 levels significantly increased (301.9±101.9 vs 190.4±65.4 ng/ml, $P<0.001$) and were normal in 94% of patients.

Before GH treatment, QoL-AGHDA questionnaire score was not different in patients with severe or no GHD (13.4±7.2 vs14.5±6.8). However we observed after GH treatment a significant decrease of QoL-AGHDA questionnaire score (9.7±7.8 vs 14.4±7.4, $P<0.001$), demonstrating an improvement of the quality of life.

Waist circumference (97±15.6 vs 98.9±16.4 cm), BMI (26.9±5.5 vs 27.7±5.6 kg/m^2), fasting glucose (0.84±0.08 vs 0.87±0.11 g/l), triglyderid level (1.14±0.42 vs 1.21±0.37 g/l), LDLc level (1.08±0.21 vs 1.10±0.25 g/l) and HDLc level (0.44±0.08 vs 0.46±0.07 g/l) had not significantly changed after GH treatment.

These results illustrate that it is important to diagnose GHD in patients with previous TBI, because of its high frequency and the positive effect of GH treatment on the quality of life, even in this group. Results after 1 year GH treatment including the dual-energy X-ray absorptiometry will be presented for the congress.

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Total and cause-specific mortality in patients from the KIMS database: direct evidence for a beneficial effect of GH replacement therapy on mortality in adult GH deficient patients

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Growth hormone (GH) replacement in adults with severe GH deficiency (GHD) has well established beneficial effects on the cardiovascular risk profile. Whether this is associated with reduced mortality is still unclear. The present study addresses this question by analysing mortality data in KIMS (Pfizer international metabolic database) and is based on 9610 patients (47% females), accumulating 37882 patient-years of follow-up. There were 257 recorded deaths against 255.1 expected. Overall all-cause mortality rate was not different from the population rate (standardized mortality ratio (SMR) 1.01, 95% CI 0.89–1.14). However, this ratio varied significantly with attained age at follow-up ($<$45 years: SMR 4.35, 45–69 years: SMR 0.84, $\geq$70 years: SMR 0.64). Female gender, craniopharyngioma or malignancy as underlying disease, pre-treatment with radiotherapy, and diabetes insipidus were associated with a significantly increased mortality rate. No significant association was seen for age at onset of pituitary disease, number of pituitary deficits in addition to GHD, as well as the presence of ACTH-, TSH-, or LH/FSH-deficiency. IGF-I SDS levels at one year of GH replacement therapy, reflecting the adequacy of GH treatment, were significantly associated with mortality ($P<0.0001$). Each unit increase in IGF-I SDS was associated with a 20.7% decrease of SMR. Poisson regression analyses identified gender, attained age, etiology of GHD, pre-treatment radiotherapy, diabetes insipidus and IGF-I SDS after 1 year of GH treatment as independent significant factors influencing all-cause mortality. Overall, deaths from cardiovascular and cerebrovascular disease and malignancies were most common; their rate, however, was not different from the normal population rate. In conclusion, the significant association between IGF-I SDS levels during GH treatment and mortality rates provides the first direct evidence for a beneficial effect of GH replacement on mortality in GHD patients.

P405

Performance characteristics of serum and salivary hormone quantification for luteal phase confirmation in behavioural studies

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Objective
To investigate gender differences in spatial orientation and verbal memory we conducted a study in 21 healthy young women and men (eleven women). Participating women were required to be in the mid-luteal phase of the cycle. Hormone profiling for behavioural studies is often performed in saliva samples to avoid venous blood sampling. We have compared simultaneously collected blood and saliva samples to confirm the presence of the luteal phase.
Methods
Serum and saliva samples were collected from eleven healthy women without oral contraceptive pill (age range 21–26 years) in mid-luteal phase (calculated from self-stated cycle lengths) at 0 and 2 h. Serum progesterone, estrogen, LH and FSH were determined on a Modular E170 system (Roche, Mannheim). Salivary progesterone and estrogen were determined by LIA (IBL, Hamburg).
Biochemical evaluation of the cycle phase was done according to the manufacturers' reference intervals. First-tier was progesterone concentration. If inconclusive, estrogen and gonadotropin concentrations were second-tiers.
Results
By combined judgement of all parameters 7/11 women were in the luteal phase. All 7 women were correctly classified using only the serum progesterone concentration (100% sensitivity). Non-luteal phase women (4/4) were correctly excluded (100% specificity). Salivary progesterone identified 5/7 women in the luteal phase (71% sensitivity). Non-luteal phase women (3/4) were mostly excluded (75% specificity).
Conclusion
Only in 7/11 (64%) of the participating women the presence of the luteal phase was confirmed by hormone testing. Therefore, hormone testing seems to be justified in neuropsychological research. Classification by serum progesterone alone was as good as classification by progesterone, estradiol, LH and FSH combined. Serum progesterone determination seems to be superior to salivary quantification. In our study an adjusted reference interval for salivary progesterone would improve sensitivity of the salivary progesterone assay, however specificity would not change.

P406

Malignant diseases in our patients with acromegaly

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Background
Acromegaly, a chronic disease caused by excess secretion of growth hormone (GH), has been known to reduce patients life expectancy. This increase in morbility and mortality is not only due to cardiovascular, respiratory or cerebrovascular disorders, but also to an increased risk for cancer.
Subjects and methods
The aim of this study was to describe the associated malignant diseases among our fifty acromegalic patients. Clinical features of these patients (age, gender, smoking status, Diabetes and Hypertension prevalence) were recorded, as well as relevant details concerning acromegaly (ethiology, mean age of diagnosis, therapy and metabolic control of disease). The associated neoplasm and its relation with the onset of acromegaly was also analysed.
Results
Seven (3 men, 4 women; mean age: 67 ± 12.5 year) of a total of fifty patients with acromegaly (19 men, 31 women; mean age: 56.2 ± 16.4 year) had a malignant disease. None of the seven were active smokers, two suffered from diabetes and four from HTA. Their mean age diagnosis of acromegaly was 62.3 ± 12.8 year with a mean duration of acromegalic disease of 4.6 ± 4.7 year. They had been treated with transsphenoidal surgery for their GH secreting pituitary macroadenoma and active acromegaly was found on 6 of them. Colorectal, breast and thyroid carcinoma were each observed on two different patients whereas prostatic carcinoma was recorded on one. These diseases appeared before acromegaly in five patients and during its follow-up on two.
Conclusions
In our acromegalic series, 14% have a second malignant disease with a higher prevalence of colorectal, breast and thyroid neoplasm. The first two agree with what has been reported in literature, whereas the association between thyroid carcinoma and acromegaly is still to be discussed. Large-scale studies are needed to draw conclusions relating to cancer risk.

P407

Pituitary abscess: a case report

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Pituitary abscess is a very rare entity, only 200 cases (mostly the complication of the pituitary surgery) has been described so far.
We want to present the case of pituitary abscess arising from Rathke's cyst.
Case report
Seventy-years-old female treated due to clinical depression, was admitted to the Endocrinology Department because of profound hyponatremia (serum Natrium 119 mmol/l). She was diagnosed with plurihormonal anterior pituitary insufficiency. After implementation of substitution with hydrocortisone and levothyroxin hyponatremia resolved. Pituitary MR revealed expansive tumor, hyperintensive on T2-weighted images, suggesting the Rathke's cyst. Mild

hyperprolactinaemia was attributed to the pituitary stalk compression by the tumour and the treatment with antidepressants. The patient was referred to the Neurosurgery Department, where the tumor was removed by the transsphenoidal approach. Histopathological evaluation revealed a cyst filled with non-specific granulation and fibrous tissue and planoepithelial metaplasia of the cyst wall. The tumour invaded the sphenoid sinus. The surgery was complicated by late cerebrospinal fluid leakage.
Conclusions
Pituitary tumor may be a difficult diagnostic dilemma, particularly in the absence of the general symptoms. Although sellar abscess is extremely rare, it may be suspected in patient with invasive cystic lesion in the pituitary. The correct diagnosis may be made only based on histopathological examination.

P408

Effects of weight gain on serum adiponectin, leptin and ghrelin concentrations in patients with anorexia nervosa

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Objective
The aim of this study is to determine serum concentrations of adiponectin (ApN), leptin and ghrelin in different stages of anorexia nervosa (AN) and to evaluate their relationships between biochemical, hormonal and anthropometric parameters.
Materials and methods
The study is composed of four groups. Group 1: Treatment-naïve patients with a recent diagnosis of AN (*n*: 19), Group 2: The weight recovered (10% increase in body weight compared with baseline) subgroup of group 1 at the end of a mean 117.72±98.14 days of follow-up (*n*: 12), Group 3: Recovered patients with a previous history of AN but normal menstrual cycles and body weight at the present time (*n*: 10), Group 4: constitutionally thin healthy young women with normal menstrual cycles (*n*: 10). Blood samples were obtained for measurements of biochemical parameters, ApN, leptin and ghrelin and body composition was determined by bioimpedance analysis.
Results
Mean age of group 1 was significantly lower compared with group 4 (19.6±3.6 years versus 24.0±2.8 years, $P<0.001$). Although serum leptin concentrations were significantly lower in group 1 patients compared with group 3 and 4 (5.6± 10.8 ng/ml; 10.9±6.9 ng/ml and 6.1±1.7 ng/ml respectively; $P<0.05$ for comparisons), leptin/fat mass (kg) ratios were significantly higher in group 1 patients compared with constitutionally thin controls (4.3±4.6 ng/ml per kg versus 1.1±0.5 ng/ml per kg, $P<0.01$). Leptin/fat mass ratio decreased significantly after weight regain in 12 patients (3.5±2.2 ng/ml per kg versus 1.7±1.4 ng/ml per kg, $P<0.05$). Adiponectin/fat mass ratio was significantly higher in group 1 patients compared with controls. No significant difference was observed among subgroups with respect to ghrelin concentrations. In the whole group leptin concentrations correlated significantly and positively with fat mass (kg), body mass index (BMI). Adiponectin and ghrelin concentrations showed significant negative correlations with BMI.
Conclusion
Increased leptin concentrations indexed to fat mass in AN patients may contribute to anorexia and may play a pathogenetic role.
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P409

Evaluation of verbal memory function in acromegalic patients treated with or without conventional radiotherapy after transsphenoidal surgery

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The impairment of cognitive function found in patients treated for pituitary adenomas has been associated with the effects of conventional radiotherapy (CR). Our aim was to compare the results of an examination of verbal memory function in acromegalic patients treated with transsphenoidal surgery (TS) alone to those obtained from patients treated with TS followed by CR. We retrospectively compared these two outcome groups and carried out a story recall test that included free and stimulated recalls, immediately and 20 minutes after auditory presentation. 66 patients, 26 men and 40 women, aged 55.2±12.4 years, with an average duration of symptoms before diagnosis of 5.1±3.7 years, were included in this study. Forty-two patients were treated only by TS and 24 received additional CR. There were no significant differences between groups in sex, age, average duration of symptoms before diagnosis, and mean GH and IGF-1 levels before TS (18.1 and 21.1 μg/l for GH, and 820.1 and 889 μg/l for IGF-1, respectively in both radiated and not radiated groups). Although there were more pituitary deficits in the radiated group when their verbal memory was assessed, the prevalence of growth hormone deficiency was similar in both groups, as well as the percentage of patients meeting cure criteria. The CR group performed significantly worse in the immediate recall, either free, with mean scores based on age-adjusted normative data of 23.4 and 39.2, for CR and not CR groups ($P=0.001$), or after questions, showing scores of 23.3 and 39.3 ($P=0.001$). These differences increased in the delayed recall, where mean scores were 20.6 and 40.2 for free recall ($P<0.0001$), and 18.1 and 42.2 after questions ($P<0.0001$). In our study, postoperative CR in patients with acromegaly is associated with a decrease in verbal memory function when compared to TS alone.

P410

The role of inferior petrosal sinus sampling for ACTH-dependent Cushing's syndrome

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Objective
Inferior petrosal sinus sampling (IPSS) with CRH stimulation is considered the gold standard for the differential diagnosis of ACTH-dependent Cushing's syndrome. The aim of this study is to evaluate its role in confirmation and lateralisation of a pituitary source in suspected Cushing's disease (CD).
Methodology
Nine patients with CD with indeterminate MRI underwent IPSS from August 2004 to February 2007. Three were being assessed for recurrent disease. The following parameters were evaluated: i) Central- to- Peripheral ACTH gradient for localisation, ii) Inter-petrosal sinus gradients for lateralisation, iii) Surgical histopathology, and iv) Post-operative clinical course.
Results
A Central- to- Peripheral ACTH gradient of ≥2.0 was found in 7/9 patients at base line and a gradient of ≥3.0 in all patients after CRH stimulation, confirming pituitary source of ACTH excess. Inter-petrosal sinus gradient of ≥1.4 was observed in 8/9 patients at base line and in all patients after CRH stimulation. This correlated with lateralisation of the excess ACTH source in all patients undergoing their first resection resulting in remission. For recurrent CD patients, the site of resection was made intra-operatively based on macroscopic appearance and frozen section. The IPSS lateralisation did not appear to have influenced the decision. Two had pituitary adenoma confirmed, however all three did not achieve remission.
Conclusion
IPSS can help confirm the location and even lateralise the source of ACTH production. In patients undergoing their first resection, the results are useful to direct surgeons to the appropriate site of excision (100% sensitivity and specificity). For patients with recurrent disease, IPSS was useful in confirming the source of excess ACTH. However, its role in determining and guiding the site of surgical excision still warrants further investigation.

P411

Comparison of visceral adipose tissue content between diabetic and nondiabetic acromegalic patients

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Acromegaly is known to be associated with insulin resistance. Visceral adipose tissue is increased in patients with insulin resistance and we aimed to investigate a visceral adipose tissue content as a possible marker of insulin resistance in

diabetic and nondiabetic acromegalic patients. We compared 16 diabetic and 16 nondiabetic acromegalic patients for fasting blood glucose (FBG), insulin and visceral adipose tissue content calculated from abdominal computer tomography. All patients were active acromegalic. Diabetic patients were well controlled with diet alone or diet and oral antidiabetic drugs. Diabetic patients were significantly older than nondiabetics(51.94 ± 10.17 vs 41.38 ± 13.43 years, $P = 0.018$). GH and IGF-1 levels were similar in both groups. Visceral adipose tissue content did not differ between diabetic and nondiabetic patients. Age, GH, IGF-1, FBG and fasting insulin were not correlated with adipose tissue content. In conclusion, concomittant well controlled diabetes might not affect visceral fat content in acromegalic patients and this might be at least partly explained by high GH and IGF-1 levels.

P412

A non-functioning pituitary carcinoma with cerebellar metastases

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Pituitary carcinomas are rare but represent a particular challenge to clinical practice. We report a case of pituitary carcinoma with metastatic disease in a separate non-contiguous foci within the central nervous system. A 64 year old man presented with a large non-functioning pituitary adenoma manifesting as headache, third pair of cranial nerves paralysis, panhypopituitarism and impaired visual acuity in 1996.The tumour was grossly removed through a right pterional craniotomy. Conventional radiotherapy for the residual tumour was performed in 1997. Follow-up magnetic resonance (MR) imaging revealed a residual tumour close to the left cavernous sinus and in the posterior sellar region. New signs of mass effect with headache, ataxia and dismetria developed in 2006. A MR showed a tumour located in the cerebellar vermis, which was excised by suboccipital craniotomy. Histological examination of the specimen revealed typical pituitary adenoma. The post-surgical MR revealed only a residual tumour in the sellar region. Other studies including chest radiography, abdominal ultrasound, vertebral column MR, octreoscan and positron emission tomography (PET) did not show any other metastases. Conclusions: this case documents an 11 year history of a rare non-functioning pituitary carcinoma which metastasized in cerebellum and shows that pituitary carcinomas may present as typical pituitary adenomas which may reveal their malignant character with distant metastases only as time progresses. Distant lesions should be removed for histological evaluation to plan the subsequent management.

P413

Impact of hyperglycaemia on carotid intima-media thickness in acromegaly

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Acromegaly is known to be associated with both increased cardiovascular mortality and carbohydrate intolerance. The aim of our study is to compare intima-media thickness, a marker of atherosclerosis in active acromegalic patients with and without diabetes. 32 active acromegalic patients (M/ F: 15/17, 16 diabetic and 16 nondiabetic) were assessed for fasting blood glucose (FBG), insulin, growth hormone, insulin-like growth factor 1 and carotid intima-media thickness (IMT). Diabetic patients were significantly older than nondiabetic patients (51.94 ± 10.17 vs 41.38 ± 13.43 years, $P = 0.018$). Diabetes was diagnosed after the onset of acromegaly in all subjects in diabetic group and well-controlled with diet and/ or oral antidiabetic drugs. IMT was significantly higher in acromegalic patients with diabetes compared to nondiabetic group (0.72 ± 0.16 vs 0.60 ± 0.08 mm; $P = 0.018$). GH and IGF-1 levels were similar in both groups. In whole group, age and FBG was correlated significantly with IMT ($r = 0.438$; $P = 0.015$ and $r = 0.444$; $P = 0.014$, respectively), GH, IGF-1 and fasting insulin were not. In conclusion, advanced age and hyperglycemia seems to have a significant impact on the progression of atherosclerosis in the course of acromegaly.

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Autoimmune hypophysitis and isolated corticotrophin deficiency

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Introduction
Isolated ACTH failure is a rare disorder characterized by a secondary adrenal failure and a considerable deterioration of the general state. In adults the has been established related to cranial traumatism and to autoimmune hypophysitis (AH). It has been pointed out that the isolated failure of pituitary tropin in the context of a AH, could end with a global pituitary failure.
Objective
Evaluation of the shortage of isolated ACTH as a way of presentation of the AH.
Materials and method
There will be described two cases of selective failure of ACTH (cases 1 and 2), with an acute beginning of the symptoms, and its development along time. They will be compared with three cases of HA with combined hormone secretion alterations (cases 3, 4 and 5) with a more silent course.
The nomination of the pituitary antibodies was carried out by indirect immunofluorescence technique, using human pituitary (cases 3 and 4) or primates ones (cases 1, 2 and 5).
Results

Cases	Age	Sex	Auto-immune thyroid disease	Years of control	MRI pituitary	Clinical features
1	33	F	yes	1	pituitary gland normal	Syncope, hypoglycemia amenorrheoa
2	40	F	yes	5	pituitary gland normal	General fatigue, headache, amenor-rheoa
3	40	M	no	15	pituitary gland normal	Polyserositis, decreased libido and potency
4	28	F	no	11	symmetric enlargement	General fatigue, ame-norrheoa, headache
5	37	F	no	19	symmetric enlargement	Headache, amenor-rheoa

Cases	FT_4 ng/dl	TSH μ/ml	PRL ng/ml	GH ng/ml	IgF-1 nmol/l	ACTH pg/ml	Corti-sol nmol/l	LH μ/ml	FSH mU/ml	E pg/ml	T ng/ml
1	0.94	8.8	12.5	1.5	54	13.6	46	8	9	23	
2	0.97	12	4	0.2	7	27	271	3	9.7		
3	0.48	4.1	3.7	1.4	4.20	1	120	0.74	1.4		34
4	0.85	0.620	54	1.3	13	487	0.20	0.57	0.20		
5	0.62	1.87	41	1.13	10	15.7	333	0.2	2.4	0.54	

Conclusion
The isolated failure of ACTH is able be the way of presentation of an autoimmune hypophysitis, although the disease could or could not turn into a panhypopituitarism. In this case this may be its debut clinic manifestation.

P415

Heel pad thickness in diabetic and nondiabetic acromegalic patients

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Increased heel pad thickness can be used as a marker of soft tissue enlargement in acromegalic patients. We aimed to investigate if concomittant hyperinsulinemia/and or diabetes secondary to insulin resistance in acromegaly contribute to the enlargement of heel pad. We compared heel pad thickness in 16 diabetic and 16 nondiabetic active acromegalic patients using X-ray images. All of the diabetics were well controlled with diet and/ or oral antidiabetic drugs. GH and IGF-1 were similar in both groups. Heel pad thickness did not differ between diabetic and nondiabetic acromegalic patients (21.93 ± 3.54 vs 22.92 ± 3.80 mm, $P = 0.489$) and was not correlated with fasting blood glucose, insulin, GH and IGF-1. In conclusion, diabetes secondary to acromegaly did not affect soft tissue enlargement represented by heel pad thickness.

P416

Perioperative steroid treatment is not routinely required in endoscopic transphenoidal surgery for clinically non functioning pituitary adenomas (NFPA)

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Steroids are still widely prescribed in pituitary trans-sphenoidal surgery (TSA). Pituitary-adrenal/thyroid/gonadal functions were prospectively evaluated in 72 consecutive NFPA pts (20–87 years, 37 M) before and after endoscopic-TSA (E-TSA) (63 at first operation, 9 at re-operation). All had macroadenoma with suprasellar extension: impinging optic chiasma in 28, extending into cavernous sinus (CS) in 23, giant in 12. Hydrocortisone was infused peri-operatively only in pts with pre-op 0800 am cortisol (F) <8 μg/dl (arbitrary cut-off). After E-TSA clinical picture, F, electrolytes, FT_4 and diabetes insipidus (DI) were checked at 1–3 days, testosterone in M or menses/FSH in women at 1 month, ACTH 1 μg-induced F peak (F-ACTH) and MRI at 3 months. A wide resection ($>90\%$) was performed in all pts, up to empty sella in 60%. Post-op follow-up lasted 1–11 years (median 5) and regrowth occurred in 5 points with CS invasion. The greater the adenoma size, the worse the pre-op and post-op pituitary function. Central hypogonadism (HypoG) and hypothyroidism (HypoT) were detected in 80.5% and 40.3% before and in 77.8% and 47.2% of pts after E-TSA. Permanent DI occurred in 13.9%. F was 11.4 ± 3.9 before and 11.5 ± 4.3 μg/dl after E-TSA. Pre-op hypocortisolism (HypoA) did not change in 14 points (19.4%, all had also hypoT and hypoG), and was detected in 6 (10.3% of those with previously normal adrenal function) at the first post-op control. No patient whose pre-op F was >8 μg/dl failed, and no patient but 1 whose pre-op F was <8 μg/dl achieved the required 18 μg/dl F-ACTH cut-off. In conclusion, pituitary-adrenal function is usually preserved in NFPA, and only seldom is impaired after complete tumor removal by E-TSA; the first 1–3 day post-op control reveals the few cases impaired by E-TSA. We recommend peri-operative steroid treatment only In pts with pre-op subnormal F levels, and to evaluate clinical picture and morning F on post-op day 1–3 for guidance about replacement treatment.

P417

Pituitary deficiencies after autologous bone marrow transplantation

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Development in autologous bone marrrow transplantation (auto-BMT) has improved survival, but new endocrine complications now emerge. If primary thyroid and gonadal deficiencies are documented in medical literature, pituitary deficiencies are less well-known, especially in adults. The aim of the study was to investigate pituitary function in patients who survived at least one year after transplantation for malignant haematologic disorders by a prospective study.
Material and methods
Thirty patients (17 male, 13 female) were studied. Age at transplantation ranged from 7 to 67 years. Preparation of patients had included total body irradiation (TBI) in eight patients, only cytotoxic drug in 22 patients. In 17 patients, immunotherapy (interferon and interleukine-2) was used. Corticosteroid treatment had been stop for at least 6 month. Prospective hormonal analysis was effectuated, including basic dosages (TSH, T4L, IGF1, FSH, LH, E2/T, PRL), and dynamic test of hypothalamus-pituitary axis (HPA) with insulin tolerance test (ITT) on GH and cortisol or adrenocorticotropin (ACTH) test (corticotrophin 1 μg IV) if contraindication or insulin resistance.
Results
Seven patients (24.1%) had central hypothyroidism. Secondary adrenal insufficiency was diagnosed in 2 subjects. GH deficiency was diagnosed in 7 subjects (1 severe, 6 partial). Eight no menopausal women before allo-BMT had primary ovarian insufficiency. Four male presented central hypogonadism.
Sixteen patients (64%) had one pituitary deficiency, 1 had 2 deficiencies and 2 had 3 deficiencies. No global HPA deficiency was found.
Discussion
Two majors factors seem to be important for developing endocrine dysfunctions: conditioning treatment before auto-BMT: TBI and cytotoxics drugs or corticosteroid used before BMT. Considering these results, a systematic screening of pituitary deficiencies after auto-BMT could be proposed in order to improve patient quality of life after transplantation.

P418

Consequences of isolation stress on glucocorticoid regulating genes and behavioural response in postnatal pigs

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Early life events can have short and long-term effects on neuroendocrine, autonomic, and behavioural responses to stress and appear to play an important role in the etiology of stress-related disorders. Glucocorticoids are thought to be key mediators of these brain-endocrine-immune connections. We investigated the effects of a short-term social isolation (4 h) of domestic piglets at different days of age (7, 21, 35) both on behavioural alterations in open-field tests and on modifications in the expression of genes regulating glucocorticoid response in stress-related brain regions. Evaluation of glucocorticoid receptor (GR), mineralocorticoid receptor (MR), 11β-hydroxysteroid dehydrogenase 1 and 2 (11β-HSD1 and 11β-HSD2) mRNA expression by real-time RT-PCR revealed significant differences in quantity of these genes in hypothalamus, hippocampus and amygdala. The social isolation caused a significant increase in ACTH and cortisol concentrations and an increase of behavioural arousal. The increased activity of the HPA axis and the behavioural alterations were associated with distinct changes in gene expression in the limbic system. The hypothalamic GR, MR and 11β-HSD1 mRNA levels significantly increased in piglets exposed to isolation stress, whereas in the amygdala the MR mRNA expression significantly decreased. There was also a significant increase of HSD1 mRNA in isolated piglets in hippocampus on day 7 of age, but the 11β-HSD2 mRNA levels were not influenced by social isolation in the brain regions. In conclusions, psychosocial stress in form of a short-term social isolation in piglets caused age-dependent and region-specific modifications in mRNA levels of stress-related genes in the brain accompanied by changes in open-field behaviour indicating adaptive arousal and experienced distress. Therefore, we suggest that psychosocial stress effects should be considered in livestock handling practices (e.g. the weaning of piglets).

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Demography, characteristics and outcome of acromegaly at King Hussein medical centre amman Jordan

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Acromegaly is a chronic debilitating disease due to long term exposure to elevated levels of growth hormone (GH) which leads to excessive growth of both skeletal and soft tissues. It is slowly progressive with 30% increase in mortality rate for cardiovascular disease, respiratory complications and malignancies. The estimated prevalence of the disease is 40 cases per million with 3–4 new cases per million populations per year. Acromegaly most commonly presents between the third and fifth decade with 6–10 years delay in diagnosis. Effective treatment can improve the survival to that of aged matched population. In 2003 a core of neuroendocrie-pituitary clinic was established at KHMC with a central referral unit for all satellite army hospital across the county.
Aim
To assess the outcome of medical and surgical treatment of our group of Acromegalics and to evaluate predictor factors of cure.
Patients and methods
Fifty one patients with acromegaly were studied; one elderly patient refused all modalities of treatment. Random GH and mean GH to OGTT, IGF1, and IGFBp3 were assessed prior and after treatment as well as radiological findings. Age, duration of acromegaly, body weight, skin fold thickness and tumor size were evaluated as predictors of favorable outcome.
Results
There were 28males and 23 females with mean age of 43.4 ± 11.9 years (range 24–74). The duration of disease was 7.7 ± 8.7 years and of follow up was 9.4 ± 8.55 years (0.5–22). Ninety percent of patients were having macro adenomas. The mean GH to OGTT (pre versus post op) was 19.7 ± 13.9 ng/ml versus 4.4 ± 7.8 ($P<0.0003$), IGF1 was 815.5 ± 303.7 versus 415.1 ± 326.7 ($P=0.016$) and IGFBp3 is 7.6 ± 2.7 vs 5.6 ± 2.2 mg/ml ($P=0.049$). One third of patients achieved cure the rest is still active. Seventeen patients are medically treated by Somatostatin analogues, 16.7% of whole group achieved cure on treatment. Eight patients received radiotherapy; 50% of them achieved cure. Mean HGH for the group receiving medical treatment (SSA) was 20.33 ± 12.71 ng/ml versus 8.11 ± 10.2 post follow up period. A GH of <15 ng/ml and age <40 years were found to be the strongest predictors of favorable outcome in this cohort.

Conclusions
Cure rate is modest when compared with international rates. Younger age group and lowest growth hormone levels are the strongest favorable predictors to cure. Our patients are presenting late with aggressive tumor which limit the cure rate. Medical therapy achieved acceptable cure rate and plays an important role as adjunctive therapy after surgical resection of pituitary tumor. A multidisciplinary approach toward acromegaly management is recommended.

P420

Sleep quality in patients of a primary health care unit and its relationships with antropometric parametters

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Objective
To study the sleep quality in patients of a primary health care unit and Its relationship with anthropometric parameters as sex, age, BMI and waist circumference.
Participants and methods
One hundred and twenty-two patients arrived spontaneously and correlatively for consultation. The Pittsburgh sleep quality index questionnaire (PQ5I) was used to investigate the patients sleep quality in the last month. The PQ5I explores seven aspects: C1) subjective sleep quality; C2) sleep latency; C3) sleep duration; C4) sleep efficiency; C5) sleep disturbances; C6) use of specific medication; C7) daytime disturbances. The PQ5I provides a score (0-3) of these seven components and a global PQ5I score in a (0-21) scale. A higher score indicates poor sleep quality.
Statistical study
Descriptive statistic, ANOVA and Pearson correlation was used to value the relationship with anthropometric parameters.
Results
Sleep quality
PQ5I global score indicates that 52.1% of the patients are 'good sleepers', the others display sleep disturbances in some components. Components: C1) 73% of patients considers that their sleep is good or very good; C2) 17.3% needs more than one hour for sleep onset; C3) the sleep hours average were 6–7 h per night; C4) 68% have a good sleep efficiency; C5) for the 91%, of them present some sleep disturbances; C6) 12% need sleep medication every day; C7) for the 90%, their sleep quality does not entail any diurnal activities dysfunction.
Sleep quality and anthropometric measures
Patients sleep quality deteriorate with age, being more evident in women. Poor sleepers, have greater BMI and waist circumference. The relationship among poor sleep quality, less sleep hours and BMI is more evident in women, whereas this same relationship related to the waist circumference is more important in men.
Conclusions
In our patients poor sleep quality and total sleep hours reduction are associated to higher BMI and waist circumference.

P421

Glucose-dependent insulinotropic polypeptide receptor (GIPR) in human pituitary adenomas

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Glucose-dependent insulinotropic polypeptide (GIP) receptor is a member of the G protein-coupled receptors family. According to its incretin properties, GIPR is expressed in tissues such as pancreas, stomach and adipose tissue. However GIPR expression pattern appears to be broader, as it was detected in other tissues such as heart, lung and the central nervous system, thus suggesting either novel ligands for GIPR or novel actions for GIP. Moreover, in some patients with food-dependent Cushing's syndrome, ectopical expression of GIPR was demonstrated being sufficient for inducing symptoms of hypercortisolemia and the formation of a benign adrenocortical tumor.
For such reasons, and in order to establish its possible involvement in pituitary adenomas tumorigenesis, GIPR expression was investigated in a large cohort of pituitary adenomas.
The study population consisted of 42 pituitary adenomas obtained after transsphenoidal surgery and 12 normal pituitary glands with autoptic origin (NPG). Based on the hormonal pattern and immunohistochemical studies 9 adenomas were classified as corticotropinomas, 12 were somatotropinomas (GHomas), 6 prolactinomas and 15 non-functioning pituitary adenomas (NFPAs).
Expression studies demonstrated that 5/12 GHomas and a single NFPA showed high GIPR expression compared to NPG in which low levels were detected. Similarly, low levels were observed in all other samples. The evaluation of clinical and biochemical parameters of acromegalic patients, revealed that for the only three high-GIPR expressing subjects, for which the OGTT was available, a paradoxical increase of GH after oral glucose load was observed. Such an effect was never observed in low-GIPR expressing subjects.
This data together lead us to hypothesize that in GHomas, GIPR may be possibly involved in the mechanism of GH-regulation exerted by the glucose, whereas it probably does not play any role in the pathogenesis of the other pituitary adenomas. Further studies are mandatory to establish the real role of GIPR in acromegaly.

P422

Macroprolactinaemia in men

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Background
It is known women suffer from macroprolactinaemia more frequently than men. Often men with unclear genesis of hyperprolactinaemia have normal or a little depressed Testosterone level. High macroprolactin level in blood serum can explain this type of hyperprolactinaemia.
Materials and methods
Sixty-four men with hyperprolactinaemia (Prl >600 mU/l) were studied. Clinical, biochemical and MRI methods were used. Prl and Testosterone levels were determined by fluorescence method. MonPrl was determined by PEG-precipitation (Delfia; Finland). Macroprolactin level was calculated from Prl and monPrl data. After investigation of 35 healthy men with normoprolactinaemia reference value were estimated: 74–390 mU/l. A recovery of macroprolactin more than 60% was accepted as macroprolactinaemia.
Results
Macroprolactinaemia was founded in 11 (I group) of 64 cases (17.1%). True hyperprolactinaemia was founded in 53 (II group) of 64 cases. All patients of II group have been taken cabergoline (median of dose – 2.5 mg/week). Median of Prl level in I group was 2356 mU/l. Median of Testosterone level was 10.4 nmol/l. The microadenomas were revealed in 3 men, macroadenomas – in 6 patients. Clinical symptoms (libido impairment, reduced sexual potency) were founded in 9 men, they all had increased monPrl level (978 mU/l). That is why all of these patients have been taken cabergoline treatment (Median dose-1.25 mg/week). In 2 patients from I group monPrl level was normal and dynamic control carried out. After 6 months median of Prl level was 689 nU/l, testosterone level-14.1 nmol/l. There were no clinical symptoms in all 9 men. monPrl level was normal in all patients (median-265 mU/l). Positive dynamics of the tumors volume was noted in 5 patients with macroadenomas (from 2.5 to 1.9 cm^3) and in 2 patients with microadenomas (from 0.9 to 0.5 cm^3).
Conclusions
This investigation revealed that macroprolactinaemia is founded in 17.1% in men with tumorous and not tumorous hyperprolactinaemia. MonPrl level was higher than reference value in 9 of 11 patients with macroprolactinaemia and they were treated by cabergoline. But cabergoline dose in I group was greatly lower than in II group. Therefore efficiency estimation should be determined on monPrl level, not on Prl level.

P423

Age-dependent expression of ret receptor from perinatal life to adulthood

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The RET/GFRa/GDNF system displays a wide distribution in tissues such as nervous system, kidneys, intestine or testis. RET has been implicated in human

pathologies. RET is a tyrosine-kinase receptor activated by four ligands GDNF, NTN, ART and PSPN through four co-receptors GFRa1, 2, 3 and 4 respectively. Activation of the receptor leads to cell differentiation or proliferation. Our group has shown that RET is expressed specifically in the somatotrope (GH) adult cell population within the pituitary, both in rats and in humans (Urbano, Endocrinology2000; Japon, JCEM2002) and provided evidence that RET expression controls the number of somatotropes *in vivo* inducing apoptosis (vs survival) through overexpression of Pit-1 (Cañibano, EMBOJ2007).
Here we characterize the mRNA levels of RET receptor by q-RT-PCR during the maturation of the pituitary from birth to adulthood.
Adenopituitaries of 24 hour, 10, 20, 30, 60 (adults) and 90 days-old rats were isolated and frozen.
Specific primers were used for: GRFa2, a positive control of inmature gland since is expressed in embryonic pituitary (Golden, ExpNeurol1999); Pit-1, essential for development of somatotropes, lactotropes and thyrotropes; GH, a control of pituitary growth maturation.
Expression of GFRa2 was high in newborn pituitaries and decreased with age. Opposite, GH levels were low during the first 10 days and they doubled its expression at 20 days around puberty remaining high through adulthood. It was observed that Pit-1 and Ret showed similar patterns of expression: both were expressed at birth at low quantities; at 10 days a peak of RET expression was seen decaying at 20 days; Pit-1 also increased its expression at 10 days but peaked at 20 days to decay later.
In summary, during growth expression of RET precedes a Pit-1 surge of expression just before the GH raise characteristic of puberty. This suggests that RET controls somatotrope cell generation in the pituitary.

P424

Midnight salivary cortisol (MSC) to assess the outcome of transsphenoidal surgery (TSS) in Cushing's disease (CD)

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Introduction
MSC is a simple and reliable mean to diagnose hypercortisolism, yet its value to asses the outcome of treatment has rarely been addressed.
Objective
Compare MSC and other classical parameters, to assess the outcome of TSS in CD.
Patients and methods
Sixty-eight patients from a single Center operated for CD between 1996 and 2006. Outcome was assessed between 6–12 months post TSS. Remission was defined as: morning plasma cortisol <50 ng/ml and/or insufficient response to ACTH; or normal urinary free cortisol (UFC <90 µg/d) together with midnight plasma cortisol <75 ng/ml and normal suppression to dexamethasone. Morning plasma cortisol at day five post TSS (TSS $+5$) was also retrospectively analysed.
Results
The 73.5% of the patients achieved remission. MSC was significantly lower remission group (group 1, $n=50$) than of failure (group 2, $n=18$): 0.7 ± 0.4 ng/ml (mean $\pm$s.d., range: 0.4–2.2 ng/ml) versus 6.5 ± 6.6 ng/ml (range: 2.1–27.2 ng/ml), $P<0.001$. Similarly, UFC was significantly lower in group 1 than in group 2: 14 ± 19 µg/d (range: 2–105 mg/d) versus 307 ± 304 µg/d (range: 20–1070 µg/d), $P<0.001$. Retrospectively, morning plasma cortisol at TSS $+5$ was lower in group 1 than in group 2: 26.8 ± 46.4 ng/ml (range: 10–270 ng/ml) versus 136.6 ± 85.6 ng/ml (range:14–298 ng/ml), $P<0.001$. MSC was correlated to UFC (r 0.618, $P<0.001$), as well as to morning plasma cortisol at TSS $+5$ (r 0.410, $P<0.001$). For the diagnosis of remission the performances (specificity-sensitivity) of the different measurements were as follows: MSC (cut off of 2 ng/ml) 100%–98%; morning plasma cortisol post TSS $+5$ (cut off 18 ng/ml) 92.9%–26%; UFC (cut off of 90 µg/day) 70.6%–21%.
Conclusion
MSC is a valid marker of remission after TSS for CD, with better sensitivity and specificity than UFC or early morning plasma cortisol immediately after TSS.

P425

Tests of growth hormone (GH) status in severe GH deficiency: do they identify a similar phenotype? Insight from the KIMS database

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A GH peak of 3 µg/l during the insulin tolerance test (ITT) is considered the gold standard for identifying adults with severe GH deficiency. Alternative stimuli such as arginine (AST) and glucagon (GST) are also employed but produce lower GH peaks than the ITT in normal subjects. Despite this, 3 µg/l is used as the diagnostic threshold for these tests, raising the possibility that severe GH deficiency is being diagnosed inappropriately.
We studied 4584 patients enrolled in KIMS (3070 underwent an ITT, 1320 an AST and 910 a GST at diagnosis) to determine whether these patients exhibited a similar phenotype. Interquartile ranges of GH peaks achieved during the ITT were determined (1st <0.12, 2nd 0.13–0.39, 3rd 0.4–0.99, 4th 1.0–3.0 µg/l). These limits were applied to the AST and GST. Comparisons were made in the 1st and 4th quartiles between the AST vs ITT and GST vs ITT for the following clinical variables: BMI, IGF-I SDS, cholesterol, LDL-cholesterol, HDL-cholesterol, triglycerides, waist:hip ratio and quality of life (QoL-AGHDA). quartile. AST vs ITT: BMI was higher (30.6 vs 28.8 kg/m2, $P<0.0001$) in the AST group. GST vs ITT: IGF-I SDS was lower (-1.9 vs -2.8, $P=0.003$), HDL was higher (1.1 vs 1.2 mmol/l, $P=0.025$) and triglycerides were lower (2.7 vs 2.3 mmol/l, $P=0.003$) in the ITT group. quartile. AST vs ITT: BMI was higher in the AST group (29.0 vs 28.0 kg/m2, $P=0.035$). GST vs ITT: Quality of life was worse in the GST group (15 vs 12, $P<0.0001$).
None of the other parameters was significantly affected in either quartile.
Using a diagnostic threshold of 3 µg/l, the AST and GST identified GHD patients with similar features to those defined by the ITT. This is reassuring for future studies on the impact of GH replacement in the KIMS database.

P426

Enos, P22^{phox} and apoE gene polymorphism associations with circulating hormone levels in the elderly

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Objective
The aim of this study was the identification of eNOS (G894T), p22^{phox} (-930 A/G) and apoE gene polymorphisms associated with the impact of age-dependent endocrine changes.
Subjects and methods
Subjects, both genders, were recruited and classified into the three groups: (1) 100 subjects aged 55–80+ years with mild cognitive impairment (MCI) (MMSE <28); (2) 165 age-matched subjects without cognitive impairment (MMSE >28); (3) 93 healthy subjects under 55 years. Serum cortisol, 17-OHP, DHEA, DHEAS, androstendion, estradiol, estrone, testosterone, free testosterone, DHT, SHBG, inhibin A and inhibin B, LH, FSH, Prl, GH, IGF1 were measured. Gene polymorphisms were assayed by using RFLP technique.
Results
The frequency of genotypes and alleles of eNOS and p22^{phox} in studied groups were not significantly different. Distribution of ε2 allele of apoE gene was higher in MCI (28.3%) as compared with healthy ones (14.6%) or adults (9.6%). Rare allele apoE ε4 presented a higher frequency in MCI (17%) as compared with healthy ones (12.5%). Raised plasma cholesterol and triglycerides were found in ε2 and ε4 carriers in MCI. In the group of adult subjects the eNOS polymorphism was significantly associated with cortisol ($\tau^2=10.36$; $P=0.006$), DHEA ($\tau^2=7.62$; $P=0.02$) and DHEAS ($\tau^2=6.94$; $P=0.03$. In the elderly, the eNOS variant correlated significantly with BMI ($\tau^2=6.47$; $P=0.04$) and FSH ($\tau^2=6.93$; $P=0.03$); p22^{phox} was associated at the border of significance with

DHEAS ($\tau^2=5.68$; $P=0.058$) and ADION ($\tau^2=5.84$; $P=0.054$). Multivariate regression analysis for eNOS G894T in the presence of associated variables showed that DHEA (OR=0.82; 95%CI=0.69–0.98; $P=0.032$) and FAI (OR=0.95; 95%CI=0.9–1.0; $P=0.034$) were associated with age.
Conclusions
The frequency of $\varepsilon 2$ and $\varepsilon 4$ variants of apoE gene raised in the elderly, particularly in those with MCI. The correlation between DHEA with age seemed to be influence by the presence of both T allele of eNOS G894T and G allele of $p22^{phox}$.

P427

Is increase in bone mineral content caused by increase in skeletal muscle mass/strength in adult patients with growth hormone (GH) treated GH deficiency? A litterature analysis

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Growth hormone (GH) has a well known anabolic effect on skeletal muscles, and patients with GH deficiency (GHD) are characterised by a reduced muscle mass, but also reduced bone mineral density (BMD) and content (BMC), which have been ascribed to GHD *per se*. Correspondingly, the increase in bone and muscle mass by GH therapy has been seen as separate GH influences on bones and muscles, respectively. The aim of this study was therefore to investigate if changes in BMD/BMC in adult patients with GHD could be due to a muscle modulating effect, and if treatment with GH would primarily increase muscle mass and strength with a secondary increase in BMD, thus supporting the current physiological concept that mass and strength of bones are mainly determined by dynamic loads from the skeletal muscles.
A literature review resulted in 34 clinical trials, published during the period 1996–2006, and fulfilling the criteria for investigating variables related to the development in muscle mass, muscle strength, and BMC/BMD in adult GHD patients treated with GH.
Three out of four trials found significant increases in muscle mass compared to baseline, while eight out of 11 trials found significant increases in one or more measures of muscle strength compared to baseline. The largest increase in muscle mass was observed during the first 12 months of treatment. Nineteen out of 26 trials found significant increases from baseline values in BMC or BMD in one or more body regions. The significant increases in BMD and BMC occurred after 12–18 months of treatment, i.e. usually later than the increase in the muscle measurements. Only 5 clinical trials studied both muscle and bone variables concomitantly in GH treated adult GHD patients. None of these studied the relationship between the changes in muscle and bone measurements.
In conclusion, although *in vitro* studies have shown that GH has a direct effect on bone remodelling, current physiological concepts and the results of clinical trials from the past 10 years suggest that the anabolic changes in muscle mass and muscle strength may also contribute to changes in BMC and BMD in the GH treated adult GHD patients. Studies addressing this particular issue are, however, needed to clarify the relative contribution of mechanisms.

P428

Neuroendocrine effects of citalopram, a selective serotonin re-uptake inhibitor (SSRI), during lifespan in humans

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Central serotoninergic activity (CSA) is known to influence the hypothalamus–pituitary–adrenal (HPA) axis, and a CSA loss seems to play a role in human brain aging and in etiology of functional hypercortisolism and depression, whose incidence increase with advancing age. Citalopram (CT), a SSRI, has been considered a good tool to evaluate CSA in humans. Aim of this study was to evaluate the neuroendocrine response to CT in healthy adult subjects during lifespan. We evaluated ACTH, cortisol (F), DHEA, PRL and GH secretion following placebo or acute i.v. CT infusion (20 mg over 120 min) in 12 young (YA, age: 29.2±1.7 years), 10 middle aged (MA, 53.2±1.3 years) and 12 elderly subjects (ES, 69.3±0.9 years). Blood samples were taken every 15′ for 240′. During placebo, ACTH, F, PRL, and GH levels were not different in the three groups, while DHEA secretion showed a clear age-dependent reduction, starting from middle age (AUC, mean±SEM, YA: 2420.7±205, MA: 938.3±94.4 μg/l per min, $P<0.01$ versus YA; ES: 674.5±62.5, $P<0.03$ versus MA). In YA, CT infusion was followed by significant increase in ACTH and F ($P<0.05$ versus placebo), while no significant hormonal changes were observed for DHEA, PRL and GH. In MA, CT increased both ACTH and F ($P<0.03$ versus placebo) and the hormonal responses were higher than in YA ($P<0.05$); no significant effect on DHEA, PRL or GH was reported. In ES, ACTH and F response to CT were lower than in MA and similar to that in YA, while DHEA, PRL and GH were not modified. These preliminary findings indicate that the corticotrope response to citalopram is amplified in middle age but not in aging, a described condition of HPA hyperactivity. This suggests that the neuroendocrine effects of citalopram are clearly age-related, showing a transient hypersensitivity to SSRI during middle age.

P429

Variable effects of preproTRH(178–199) on ACTH secretion by human corticotrope tumors

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PreproTRH (178–199), a 22-aminoacid cleavage product of the TRH prohormone, has been postulated to act as an ACTH-releasing inhibitor. Indeed, *in vitro* evidence indicates that this peptide may inhibit basal and stimulated ACTH secretion in rodent anterior pituitary primary cultures and cell lines (Redei 1995, Revskoy 2001), although not all studies concur (Nicholson 1996). Aim of the present study was to test the effect of preproTRH(178–199) in human tumoral corticotropes.
Methods
Twenty-four human ACTH-secreting pituitary tumors were collected during surgery, dispersed and established in culture. After a 3–5 day attachment period, cells were incubated with 10–100 nM rat preproTRH(178–199), with 10 nM human CRH or with 10 nM dexamethasone (all from Sigma Co., St. Louis, USA) and medium samples collected after 4 and 24 h for measurement of ACTH concentrations by IRMA. ACTH secretion was standardized to % unchallenged wells (=control).
Results
A clear inhibition of ACTH secretion at 4 and 24 h was observed in 12 specimens (for 10 nM ppTRH: 70.3±4.52% control at 4 h and 83.4±5.33% control at 24 h; for 100 nM ppTRH: 70.1±3.93% control at 4 h and 84.8±4.88% control at 24 h), whereas a mild and short-lasting stimulatory effect was observed in the other tumors (for 10 nM ppTRH: 128.6±9.45% control at 4 h and 104.7±4.26% control at 24 h; for 100 nM ppTRH: 108.4±3.42% control at 4 h and 103.3±3.63% control at 24 h). Interestingly, the effect of preproTRH(178–199) was significantly correlated with the potency of dexamethasone inhibition while no association with CRH stimulation was observed.
Conclusions
Our study in a large series of human corticotrope tumors shows that preproTRH (178–199) exerts variable effects on tumoral ACTH secretion, closely linked to sensitivity to glucocorticoid negative feedback. Further studies might shed light on its usefulness as an inhibitor of ACTH secretion by human corticotrope adenomas.

P430

Endocrine features of aging and cognitive function in women

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Objective
An on-going epidemiological populational study examined hormone balance during aging in women, and – whether there was a difference in endogenous serum sex hormone levels between a postmenopausal women group with moderately cognitive impairment and healthy age-mached women group.
Subjects and methods
One hundred and sixty-three women aged 25 to 80 years were classified into three groups: (1) 44 subjects aged 55–80 years with moderately cognitive impairment (MMSE<28); (2) 73 age-matched subjects without cognitive impairment (MMSE>28); (3-control) 44 healthy subjects under 44 years. Total morning levels of serum 17-OHP, DHEA, DHEAS, androstendion, estradiol, estrone, progesterone, testosterone, free testosterone, DHT, SHBG, inhibin A and inhibin B, LH, FSH, Prl, TSH, GH, IGF1, insulin, cortisol and thyroid hormones were measured.

Results
There was a marked decline of sex steroid metabolism associated with an increase of gonadotropins in postmenopausal women. All androgens and estrogens were significantly decreased ($P<0.001$) with no significant change in SHBG levels; gonadotropins (LH, FSH) were significantly increased ($P<0.001$). Also, metabolic balance was significantly altered. The endocrine changes were associated with a cognitive decline ($P=0.01$); serum cortisol levels and thyroid hormones showed a minimal overall change in basal levels with aging. In postmenopausal women with moderately cognitive impairment there was a decrease of serum thyroxine level ($P=0.006$) but no significant differences in estrogens and androgens levels compared to age-matched group; however, there was a higher rate in their decline.
Conclusions
Endocrine deficiencies in postmenopausal women include a decrease in the peripheral levels of estrogens and androgens with an increase in LH, FSH associated with a cognitive decline. The moderately cognitive impairment did not significantly affect mean both sex steroid and gonadotropin levels. Finally, our results emphasize the complexities of hormone action, particularly related to estrogens and androgens associated with a cognitive decline during the menopausal and postmenopausal years.

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Effect of a six-month treatment with octreotide long acting repeatable (LAR) on mean platelet volume in patients with acromegaly

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Objective
Mean platelet volume (MPV), is an indicator of platelet sizes, and an indirect marker of platelet function and activity. In this study, we aimed to evaluate MPV in patients with acromegaly, and to investigate the effects of octreotide long acting repeatable (LAR) on MPV after 6-month therapy.
Methods
Data of 25 patients with acromegaly (16 women; 9 men; mean age, 43.8 ± 13.5 years) included in the study were searched retrospectively. The data at diagnosis of the patient group was compared with a control group of 30 people similar for gender, mean age, body mass index, and co-morbid diseases. Eighteen patients of those had been unsuccessfully treated with surgery.
At the second stage of the study, the values of this patients with active acromegaly in postoperative period who receiving octreotide LAR 20–30 mg/month for at least 6 months for secondary treatment, were compared to the data of the control group and control values at 6th month of the therapy.
Results
Total cholesterol, LDL-cholesterol, triglyceride and fibrinogen levels were higher, while HDL-cholesterol levels were lower in patients than in controls. As a marker of platelet functions, when MPVs were compared, no significant difference was detected between patients with acromegaly and control group (8.90 ± 1.48 vs 8.81 ± 1.26, $P>0.05$). After 6 months of treatment with octreotide LAR in 18 patients with acromegaly, a decrease in GH, IGF-1, LDL-cholesterol, triglyceride and fibrinogen levels were observed. Also, involution rate in MPV (8.93 ± 1.25 vs $8,49\pm1.21$, $P<0.05$) were found statistically significant.
Conclusion
In this study, the first data about the effects of the disease and octreotide LAR, a long-acting somatostatin analogue, on MPV were presented. MPV is not affected in acromegaly patients, however, a decrease in MPV is observed following treatment with octreotide LAR.

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GHRH as a possible allosteric ligand of the ghrelin receptor GHS-R1a

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Ghrelin and GHRH are two hormones involved in the growth hormone (GH) secretion through their respective receptors. The growth hormone secretagogue receptor subtype 1a (GHS-R1a) is involved in biological actions exerted by ghrelin triggering intracellular second messengers coupled to $G\alpha_{q/11}$ protein. In this work, we analyzed the possible interaction between GHRH and GHS-R1a as well as the subsequent intracellular pathways. The results showed that GHRH induced, in a dose-dependent manner, a calcium mobilization from intracellular stores followed by a calcium influx through calcium channels at plasma membrane in HEK 293 cells stably transfected with GHS-R1a (HEK-GHSR1a), an effect that was not observed in untransfected HEK 293 cells. Radioligand binding and cross-linking studies revealed that GHRH-mediated response on calcium signal was mediated by GHS-R1a, showing that the presence of GHRH increased the binding capacity of ^{125}I-ghrelin. The administration of GHRH stimulated the activation of the IP_3 signalling pathway. Interestingly, GHRH potentiated ghrelin-induced IP_3 mobilization. In addition, confocal microscopy in CHO cells transfected with GHS-R1a tagged EGFP showed that GHRH was able to induce the GHS-R1a endocytosis. In conclusion GHRH is able to activate the GHS-R1a inducing an increase both in the ghrelin binding capacity and intracellular response. The regulation of the ghrelin-mediated signaling by GHRH could have implications in the GH secretion and could be an explanation of the observed synergistic effect of ghrelin and GHRH on GH secretion.

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Unusual pattern of pituitary insufficiencies following traumatic brain injury evaluated by insulin tolerance test

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There is growing evidence that pituitary failure is a common complication of traumatic brain injury. However, prevalence of hypopituitarism following traumatic brain injury varies between 25 and 69% in the literature. Moreover, the pattern of involved hypothalamic/pituitary axes differs considerably in the published trials. This might be due to small and inhomogeneous series of patients and different test methods in previous studies. The aim of our study was therefore to investigate a large cohort of patients using the gold standard insulin tolerance test (ITT). We included 90 patients at least 12 months after traumatic brain injury aged between 18 and 60 years in our trial. Patients were recruited from an out-patient clinic specialized in neuro-rehabilitation. At baseline, beside physical examination and routine laboratory testings, endocrine evaluation includes basal measurement of LH, FSH, TSH, fT3, fT4, prolactin, IGF-I, testosterone in males and estradiol in females. In addition an ITT with intravenous administration of 0.1 IU/kg body weight of regular insulin was performed in each patient. Blood samples for measurement of glucose, growth hormone (GH), ACTH and cortisol were drawn at -15, 0, 30, 45, 60 and 90 min. There was no complication during any test although all patients achieved adequate hypoglycemia. The prevalence of pituitary insufficiency is summarized in the following Table:

Pituitary failure	Diagnosed	Substituted
ACTH-deficiency	35 (39%)	23
GH-deficiency	11 (12.2%)	8
TSH-deficiency	2 (2.2%)	1
Hypogonadotropic hypogonadism	4 (4.4%)	3
Any pituitary failure	47 (52%)	35

We conclude that an ITT is a safe diagnostic tool in patients after traumatic brain injury. In 52% of these patients at least one pituitary axis deficiency was newly diagnosed. In contrast to previous studies, the predominant pituitary failure was an ACTH-deficiency followed by GH insufficiency.
We conclude that an ITT is also a safe diagnostic tool in patients after traumatic brain injury. In 52% of these patients at least one pituitary axis deficiency was newly diagnosed. In contrast to previous studies, the predominant pituitary failure was an ACTH-deficiency.

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Hypopituitarism and features of the metabolic syndrome in patients with traumatic brain injury (TBI)

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Objective
Hypopituitarism in adults may be a consequence of TBI, but in the acute or long term management of TBI patients (ptx), endocrine evaluations are not usually included. Aim of this study was to investigate the prevalence of hypopituitarism and metabolic syndrome in a population of 54 severe ($n=31$) and moderate ($n=23$) TBI ptx (38 M, 16 F; age (mean ± S.D.): 39.8 ± 2.1 years) who had TBI more than 12 months before.
Subjects and methods
The ptx were studied in a multidisciplinary team with rehabilitation doctors, neurologists and endocrinologists. The whole ptx population underwent: (1) a basal evaluation of the hypothalamic–pituitary unit; (2) the dynamic evaluation of the GH/IGF-I axis through the GHRH + Arginine test; (3) blood glucose/insuline levels (HOMA index), and lipid profile; (4) anthropometric evaluations.
Results
The 27.7% ptx showed various degrees of hypopituitarism. In particular, 9.25% had total, 7.4% multiple and 9.25% isolated hypopituitarism. Severe GH deficiency was present in 22.2% ptx, secondary hypogonadism, hypothyroidism, hypocortisolism and diabetes insipidus was present in 16.6, 12.9, 12.9 and 5.5%, respectively. In non-hypopituitaric ptx (39/54), features of the metabolic syndrome were present as visceral obesity in 35.8% ptx, dyslipidemia in 10.2% ptx, hypertension in 7.6% ptx and impairment in glucose/insulin metabolism in 33.3% ptx. If we consider only hypopituitaric ptx (15/54), features of the metabolic syndrome were present as visceral obesity in 60% ptx, dyslipidemia in 40% ptx, hypertension in 13.3% ptx and impairment in glucose/insulin metabolism in 33.3% ptx. In all, 5/54 ptx had metabolic syndrome and 4/5 were hypopituitaric.
Conclusions
Hypopituitarism and features of the metabolic syndrome are commonly diagnosed in TBI ptx. Hypopituitarism per se worsen the metabolic condition of TBI ptx, suggesting particular care of these clinical aspects in the rehabilitation period after severe or moderate TBI.

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Lymphocytic infundibulo-neurohypophysitis with diabetes insipidus and favorable evolution after glucocorticoid treatment

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Infundibulohypophysitis is an unusual inflammatory condition that affects the infundibulum, the pituitary stalk and the neurohypophisis and may be a part of a range that includes lymphocityc hypophysitis. It occurs mainly in women and most often presents in later stages of pregnancy. Infundibilo-hypophysitis usually presents with diabetes insipidus. The case of a 30-year-old woman with diabetus insipidus diagnosticated in the third trimester of the pregnancy is reported. She was still taking synthetic ADH 3 years after delivery. Evaluated in our service we found a polydipsia-polyuria syndrome (10 liters without treatment, urine density 1002), that does not respond to water deprivation test and respond to ADH administration. Sarcoidosis was excluded but the antecedents of tuberculosis and uncertain tuberculin test did not exclude a cerebral tuberculosis lesion and the diagnostic was kept in mind. Magnetic resonance imaging in these patient reveal thickening of the pituitary stalk, loss of hyperintense signal of the posterior pituitary and an adenohypophysis of normal size. After three month of corticotherapy (beginning with Prednisone 100 mg/day, 2 weeks with progressive lowering of the dose) the polyuro-polydipsic syndrome without treatment regress at 70 000 ml/day, the necessary dose of synthetic ADH dose decrease at 0.2 mg/day and the IRM imagining reveals a half reduction of pituitary stalk in all diameters. No reactivation of tuberculosis was noted after corticoids.
Conclusions
Diabetes insipidus with enlargement of pituitary stalk, with favorable evolution after glucocorticoid treatment is suggestive for infundibulohypophysitis; many of so called, 'idiopathic DI' in the past have probably autoimmune ethyology. For diagnosis a suggestive IRM imaging ± pituitary biopsy ± vasopressin-cell antibodies are necessary. A prolonged follow-up of these patients is mandatory since other autoimmune diseases can occur later.

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Prolactin levels in patients with cirrhosis increase with severity of liver disease

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Background
Pituitary prolactin secretion is physiologically supressed by dopaminergic tonus from hypothalamus, which itself is under regulation by various hypothalamic releasing factors. We Disturbed dopaminergic tonus in patients with liver cirrhosis is suspected.
The aim of our study was to measure prolactin levels in patients with cirrhosis and to investigate the relatioship between the severity of liver disease and other clinical and laboratory parameters with prolactin levels.
Patients and methods
We investigated 114 consecutive patients (74 men, 40 women) with cirrhosis. The diagnosis of cirrhosis was based on clinical and biochemical evidence and the presence of esophageal varices and/or ascites with albumin gradient > 11 g/l. Recorded variables were the routine clinical, biochemical and blood parameters, prognostic indices (Child-Pugh, MELD, variceal bleeding, portal hypertension stage) and insulin and prolactin levels.
Results
Mean age was 57 (95% CI 54.98–59.08), cirrhosis was in 77.2%, 14%, 7.8% alcohol induced, cryptogenic and viral respectively. Mean Child-Pugh score was 8.0 (95% CI 7.68–8.48), MELD score 11.4 (95% CI 10.05–12.73), 29% (95% CI 21.1–38.4%) of patients had history of variceal bleeding. Mean prolactin levels were 14.79 (95% CI 13.12–16.45) μg/l. Patients with hepatic encefalopathy comparing to patients without encefalopathy had significantly higer levels of prolactin 19.41 vs 13.93 μg/l, $P=0.017$. Prolactin levels were also significantly related to ascites degree, mean prolactin levels were 11.97 vs 15.56 vs 19.99 μg/l in patients with 1st, 2nd, 3rd degree of ascites respectively. In regression analysis prolactin levels were significantly dependent on Child-Pugh score ($P=0.016$) or Meld score ($P=0.033$). We found no impact of portal hypertension stage, gender nor etiology of cirrhosis on prolactin levels.
Conclusions
Prolactin levels increase significantly with severity of liver disease particularly in patients with ascites and hepatic encefalopathy. High prolactin level could therefore be considered as a negative prognostic marker of liver cirrhosis.

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Pituitary dysfunction in patients after stroke

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Hypopituitarism is a known complication of traumatic brain injury and subarachnoidal bleeding. There are few data about pituitary dysfunction as a complication of cerebral ischemia. This study aimed to investigate this issue. We investigated the prevalence of pituitary dysfunction in patients with cerebral ischemia (a. cerebri media: $n=19$, thalamus: $n=4$ multiinfarction syndrome: $n=17$) NIHSS varied between 1–8. 40 patients, 23 males and 17 females, (mean age f: 60 years; m: 62.5 years; range 26–81 years) with a stroke 12–34 weeks prior to the study (median 20.5 weeks) underwent clinical examination, basal and combined releasing hormone testing (CRH, GHRH, LHRH) of the pituitary gland, including TSH, free T4/T3, testosterone (males), estradiol (females), LH, FSH, cortisol, ACTH, GH, IGF 1, IGF-BP3 and depression testing. Growth hormone deficiency (GHD) was defined as a GH response < 6 ng/ml to GHRH. Secondary adrenal insufficiency was defined as cortisol response < 18.1 μg/dl to CRH. Pituitary deficiency was found in 67.5% (27/40) of the patients, 24 with one dysfunctional axis and 3 with 2–3 dysfunctional axes. 26/37 (70.3%) had GHD (13 < 65 years, 13 > 65 years). 2/40 (1 male, 1 female) had secondary hypogonadism (total testosterone < 241 ng/dl and estradiol < 30 pg/ml and low gonadotropins). 5/40 patients had corticotrope insufficiency (2 complete, 3 stress insufficiency) and none had TSH-deficiency. HADS testing was highly positive (> 10) for depression in 24/40 (60%) and positive (8–10) in 5/40 (12.5%) patients. In summary we found pituitary dysfunction in 67.5% of patients with predominantly GHD (in 13 patients maybe due to age). These findings strongly suggest that patients who suffer from stroke should routinely undergo pituitary testing.

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Abstract withdrawn

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A comparison between three automated chemiluminescence assays for growth hormone: on the use of recombinant hGH as a primary calibrant

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Objectives
GH measurements during OGTT have profound effects on therapy and follow-up management of acromegaly. To minimize the discordance between immunoassays, it is recommended to calibrate them using 22 kDa-GH-preparation instead of the use of pituitary-derived calibrants. The aim of our study was to evaluate the between-method discrepancies in GH determinations by assays using different calibrants considering further confounders like age, gender, and BMI.
Methods
GH was measured during a 75-g OGTT in 21 acromegaly patients (9 controlled; 6 men; age 34–63 years; BMI 25.8 ± 0.7 kg/m^2) and in 207 apparently healthy subjects (64 men; age 20–76; BMI 30 ± 0.5) using three Chemiluminescence-assays. Immulite2000 and Nichols were calibrated against NIBSC2ndIS98/574 for hGH, whereas LIAISON used recombinant-hGH as a primary calibrant. The Nichols-assay was re-calibrated using rhGH-preparation.
Results
The results obtained with all assays were strongly correlated in both acromegaly patients and controls ($r=0.96$ and 0.99, respectively; $P<0.001$). The results obtained with Immulite2000 were 2-fold higher than those obtained with LIAISON and Nichols; the readings of the two latter methods were in good agreement. In controls, nadir GH concentrations were (95% percentile) 0.25, 0.41 and 0.2 for LIAISON, Immulite2000 and Nichols, respectively. Using cut-off limits of 0.5 µg/l (LIAISON) and 1 µg/l (Immulite2000) identified 92–100% of patients with active disease and 67% of patients in remission.
Both basal and nadir GH levels were significantly higher in females than in males (LIAISON: 1.3 ± 0.17 vs. 0.46 ± 0.1 µg/l and 0.13 ± 0.01 vs. 0.1 ± 0.02 µg/l, $P<0.001$, respectively).
In multiple regression analysis age, BMI and gender were independent predictors for both the basal and the nadir GH levels (standardized β: -0.2, -0.3 and 0.2, respectively).
Conclusions
Post-glucose GH-nadir values are assay-, gender-, age-, and BMI-specific. The use of rhGH as a primary calibrant is advisable to reduce the between-assay differences in GH measurements.

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Acute effects of the intranasal administration of growth hormone-releasing hormone (GHRH) on nocturnal memory consolidation in waking young men

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Introduction
The activity of the somatotropic system displays a secretory maximum during early sleep which is also a period known to be important for memory consolidation. Blocking of the sleep-onset associated GH-surge by somatostatin did not affect memory performance but the contribution of growth hormone-releasing hormone (GHRH) to memory processes is unclear. Here we assessed the influence of intranasal GHRH on memory function in waking subjects during the early part of the night.
Methods
Fifteen young and healthy men (mean age 23.5 years) were investigated while staying awake throughout the night. At 21.30 hours subjects received 600 µg GHRH or placebo (saline solution) by the intranasal route in a double-blind experiment. Declarative and procedural memory was examined with a word pair learning task and finger tapping, respectively. Mood and sleepiness were measured by questionnaire (MDBF, Stanford sleepiness scale). Also hunger and thirst were monitored by a self-rating scale. Blood for determination of growth hormone, cortisol, ACTH, insulin and blood glucose were collected in close intervals.
Results
GHRH in comparison with placebo did not improve memory consolidation as expressed as difference (mean ± SEM) of recall performance between learning before substance administration (20.30 hours) and in the morning after having spent the night awake (09.00 hours): word pairs: GHRH 72.3 ± 4.1%; placebo 81.3 ± 6.4%; finger tapping: GHRH: 105.9 ± 4.7%, placebo: 99.2 ± 4.9%.
Conclusion
Memory consolidation during the early part of the night is not facilitated by the sleep associated peak activity of the somatotropic system which typically parallels this period.

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Cardiovascular risk profile in 81 acromegalic patients: a cross-sectional clinical study

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Objectives
Acromegaly is associated with elevated cardiovascular morbidity. Medical treatment of acromegaly does not only lead to a normalisation of the somatotroph axis, but can also negatively influence cardiovascular risk factors and comorbidities.
Methods
In a cross-sectional study, we evaluated cardiovascular risk markers (glucose, HbA1c, triglycerides, cholesterol, HDL, LDL, lipoprotein a, homocystein, CRP) in relation to biochemical control and medical treatment modalities in 81 acromegalic patients treated at the Endocrine Outpatient Clinic of the Max Planck Institute of Psychiatry and the Department of Medicine (Innenstadt) of the Ludwig-Maximilians-University in Munich.
Results
Biochemically controlled acromegalics ($n=47$), compared to uncontrolled patients ($n=34$), had significantly elevated total (Mean 222.2 ± 35.1 vs. 200.6 ± 41.2 mg/dl; $P=0.033$) and LDL cholesterol levels (Mean 146.6 ± 32.4 vs. 124.4 ± 40.8 mg/dl; $P=0.041$) and lower homocystein levels (Mean 11.7 ± 4.0 vs. 14.3 ± 6.8 µmol/l; $P=0.005$), but no differences in glucose, HbA1c, HDL, triglycerides, lipoprotein a, and CRP. IGF-1 correlated positively with HbA1c ($P=0.029$), and GH nadir OGTT correlated positively with HbA1c ($P=0.040$), triglycerides ($P=0.047$), and lipoprotein a ($P=0.016$), but not with glucose, CRP, cholesterol, HDL, LDL or homocystein. Somatostatin analogue (SA, $n=27$) treatment in acromegalic patients was associated with lower total (Mean 192.8 ± 30 vs. 219.0 ± 40.2 mg/dl; $P=0.016$) and LDL cholesterol (Mean 119.3 ± 25.6 vs. 143.4 ± 38.7 mg/dl; $P=0.016$), but with elevated glucose (Mean 104.7 ± 15.9 vs. 91.4 ± 16.2 mg/dl; $P=0.014$) and HbA1c levels (Mean 5.9 ± 0.5 vs. 5.6 ± 0.4%; $P=0.018$) compared to patients not treated with SA. Finally, the pegvisomant treated group ($n=10$) had lower HbA1c levels compared to otherwise treated patients (Mean 5.5 ± 0.3 vs. 5.7 ± 0.5%; $P=0.042$).
Conclusions
Our results suggest that future treatment decisions should not only target biochemical control, but a thorough evaluation of long-term consequences on cardiovascular risks depending on the different acromegalic patient profiles should additionally guide the treatment algorithm.

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Diurnal cortisol profile changes in the acute phase after aneurysmal subarachnoid hemorrhage

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Cortisol dynamics have been found to be disturbed in acute illness. Despite reports on neuroendocrine disturbances after aneurysmal subarachnoid hemorrhage (SAH), data on cortisol dynamics in the acute phase of this illness are lacking. We evaluated diurnal cortisol rhythm in n = 25 patients 1 week (t_1) and 2–3 weeks (t_2) after SAH. The study was approved by the local ethics committee. Cortisol and CBG were measured in serum, blood samples were drawn at 08:00, 12:00, 16:00 and 20:00. Clinical parameters pertaining to the severity of SAH and clinical outcome were set in relation to the cortisol measurements.
One week after SAH only 5/25 patients had normal diurnal variation of serum cortisol. 9/25 had no variation at all, 1/25 showed an inversed slope with low morning and high evening cortisol values. In 10/25 no clear pattern was detected.
Two weeks later, three of the initial 5 patients still had normal cortisol rhythms, the remaining two changed to an undetectable rhythm. 4 Patients with formerly flat rhythms and one patient without a pattern at t_1 had a normal variation at t_2. In sum, 8/25 patients had a normal circadian variation 2–3 weeks after SAH. The other patients provided no pattern (13/25) or a reversed slope (4/25). When comparing patients with normal slopes with those with no or an altered diurnal profile at t_1, those with a normal profile had a significantly less severe hemorrhage (Fisher-CT-Score; Man–Whitney *U*-test, $P = 0.042$) and a shorter hospital stay ($P = 0.052$). The same comparison at t_2 showed that patients with normal slopes gained a better outcome measured by the Glasgow Outcome Scale 3–12 months after SAH ($P = 0.039$).
First analyses suggest that a normal diurnal cortisol profile in the acute phase of SAH may be associated with a less severe bleeding and may be a predictor of a better long-term outcome.

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Pregnancy in the rat is a physiological state of leptin resistance

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Pregnancy is a state of physiological hyperphagia and represents a good model to unravel the mechanisms involved in feeding regulation. The hyperphagia is the main responsible for the positive energy balance in gestation, which is necessary for maintaining maternal energy stores. The presence of high serum leptin levels and hyperphagia during pregnancy is a paradoxical event and reflects a state of leptin resistance. In order to evaluate the mechanisms for hypothalamic leptin resistance during pregnancy, we evaluated the response of food intake, hypothalamic neuropeptides, and p-STAT3 levels to leptin administration in pregnant rats.
Diestrous and pregnant rats (13 and 18 of gestation) were used in this study. Icv and iv cannulae were implanted into third ventricle or in jugular vein respectively, five days before treatments (icv:10 μg/rat, iv:100 μg/rat). Nocturnal food intake was determined in rats fasted 6 hours before vehicle or leptin treatment. NPY and AgRP mRNA levels were determined by *in situ* hybridization using specific antisense oligodeoxynucleotide probes labeled with ^{35}S. Levels of p-STAT3 were determined by western blot in rats fasted overnight which received iv or icv leptin for one hour.
1) Pregnant rats exhibited a lack of decrease in the nocturnal food intake after icv leptin treatment.
2) Pregnant rats presented higher levels of NPY and AgRP in the hypothalamus, but we could not demonstrate a decrease in their levels after leptin treatment.
3) Administration of leptin iv and icv showed a clear increase in the levels of p-STAT3 in control rats but not in pregnant rats.
All this indicates that a reduction in leptin signalling in the hyphothalamus likely contributes to the leptin resistance mechanism during pregnancy.

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Effects of intranasal atrial natriutetic peptide (ANP) and insulin on sleep-associated pituitary–adrenal inhibition in elderly humans

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Introduction
In elderly humans sleep disturbances are associated with an impaired nocturnal inhibition of the hypothalamo–pituitary–adrenal (HPA) axis and chronic diseases like metabolic syndrome and depression. Recent experiments in young and healthy subjects demonstrated that intranasal ANP inhibited stimulated cortisol secretion during wakefulness whereas administration of intranasal insulin in the evening reduced cortisol levels in the morning after undisturbed sleep.
Methods
In a double-blind, placebo controlled experiment 19 healthy elderly (mean age 70.8 years, nine women, ten men) were treated in randomized order with intranasal ANP (1 mg) or insulin (160 IU) or placebo (saline solution) at 22.30 hours. Sleep was assessed by polysomnography and blood samples were drawn in close intervals for determination of cortisol, ACTH and blood glucose. Furthermore mood and memory as well as caloric intake in the morning after experimental nights were investigated.
Results
The cortisol nadir values were lower in subjects treated with ANP or Insulin, but differences between experimental groups were statistically not significant (mean ± S.E.M cortisol nadir (μg/dl): placebo: 2.26 ± 0.17; ANP: 1.99 ± 0.21; Insulin: 2.10 ± 0.18). No changes of mood, memory and caloric intake were detected.
Conclusion
In this sample of healthy elderly our data do not support the hypothesis that intranasal ANP or insulin improves inhibition of nocturnal HPA-activity in aged humans.

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Long-term treatment with dopamine agonists is not associated with increased prevalence of valvular heart disease in patients with prolactinomas

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Objective
Treatment with ergot-derived dopamine agonists, pergolide and cabergoline, has been associated with an increased frequency of valvular heart disease in Parkinson's disease. The aim of the present study was to assess the prevalence of valvular heart disease in patients treated with dopamine agonists for prolactinomas.
Design
Cross-sectional study.
Patients
We performed conventional two-dimensional and Doppler echocardiography in 78 consecutive patients with prolactinoma (mean age 47 ± 1.4 years, 26% male, 33% macroprolactinoma) treated with dopamine agonists for at least 1 year (mean 8 ± 0.6 years) and 78 control subjects (matched for age, gender, body surface, and left ventricular systolic function). Patients were classified according to treatment: patients treated with ergot-derived dopamine agonists (cabergoline, bromocriptine, and terguride) (group 1: $n = 57$), and patients treated with non-ergot derived dopamine agonist (quinagolide) or other treatment modalities than dopamine agonists (group 2: $n = 21$).
Results
Clinically relevant valvular heart disease was present in 12% (9 of 78) of patients vs. 17% (13 of 78) of controls ($P = 0.141$), and in 14% (8 of 57) of patients treated with ergot-derived dopamine agonists vs. 5% (1 of 21) of patients treated with non-ergot derived dopamine agonists or no dopamine agonist ($P = 0.483$). Mild regurgitation of the tricuspid valve was significantly more present in patients compared to controls (41% vs. 26%, $P = 0.042$).
Conclusion
Long-term dopamine agonist therapy in patients with prolactinoma is not associated with increased prevalence of clinically relevant valvular heart disease.

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Expansion of grey and white matter volumes and increased white matter gliosis in acromegaly: a clinicoradiological study of 43 patients

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Background
In acromegaly, few *in vivo* data exist on changes of the brain parenchyma as a pathology caused by systemically elevated levels of GH and IGF-1. This is in contrast to neuropsychiatric symptoms such as depression, cognitive impairment, personality changes and pain syndromes that accompany the condition. We aimed at measuring brain tissue volumes and white matter pathology in acromegaly.
Methods
Forty-three patients (age 54±14 years, 54% female, age at diagnosis 46.9±14 years) and age/gender matched, neuropsychiatrically healthy controls (medical comorbidities tolerated) underwent high resolution clinical MRI. Total volumes of grey matter (GM), white matter (WM) and cerebrospinal fluid (CSF) were calculated using SPM5. Raw volumes and volumes normalized to total inracranial volume were compared. Focal white matter lesions (WMLs, mainly reflecting gliosis) were rated on fluid attenuated inversion recovery (FLAIR) and T2-weighted images, employing a classification into grades 0: no WMLs, I: 1–5, II: 6–20, III: 21–40, IV: >40 WMLs.
Results
Patients showed larger absolute (627±79 vs. 604±85 ml, $P=0.076$) and normalized GM volumes (42.1±2.9% vs. 41.5±3.1%, $P=0.099$) and WM volumes (460±64 vs. 435±61 ml, $P=0.090$), and 30.8±29.8%, $P=0.043$). Absolute and relative CSF was decreased in patients (403±74 vs. 416±70 ml, $P=0.029$; 27.1±4.2% vs. 28.7±4.5%, $P=0.018$). The proportion of patients with intact WM was lower (18.6% vs. 39.5%, $P=0.028$) with most prominent shifts to WML grade I changes (44.2% vs. 30.2%).
Discussion
We provide evidence of increased GM and WM volumes at the expense of CSF in acromegaly. These changes may relate to the chronic exposure to GH and IGF-1 and its trophic effects. The reported WM pathology likely reflects the increased prevalence of vascular and metabolic comorbidities. These changes might contribute to an altered pattern of neuropsychiatric comorbidities in this patient group.

P447

GH response to GH-releasing hormone–arginine (GHRH+Arg) is impaired in women affected by human immunodeficiency virus (HIV)-related lipodystrophy

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Context
Lipodystrophic HIV patients frequently display impaired pituitary GH response. Objective. To investigate the GH response to GHRH+Arg in women with HIV-related lipodystrophy (HARS) compared with healthy females. To identify possible predictive factors for such a GH response in these patients.
Patients
Forty-nine female patients with HARS were compared with 10 healthy women matched for age and BMI.
Methods
Patients and controls underwent a standard GHRH+Arg test; demographics, anthropometric, metabolic and hormonal variables were evaluated. Basal serum GH, IGF-1, IGFBP-3, glycaemic-lipid profile, GH after GHRH+Arg and HIV disease characteristics were assessed. Body composition was evaluated by DXA in patients and controls, abdominal CT scan being performed only in the HIV patients.
Results
Using cut-offs of 4.2 and 5 ng/ml, 6.12% of the HIV-infected patients failed to reach GH peaks above these values, the percentage increasing to 22.44% with a threshold of 7.5 ng/dl; none of the controls showed a GH peak less than 7.5 ng/dl. IGF-1 was significantly lower in the HIV-infected patients with a GH peak <7.5 ng/dl than in patients with a GH peak >7.5 ng/dl and healthy females. Total lean body mass was the most significant predictive factor for GH peak after GHRH+Arg ($r^2=0.13$), followed by age in the HIV-infected females, while age was the only predictive factor ($r^2=0.46$) in the controls.
Conclusions
The study suggests that relative GH deficiency is common among females with HARS if compared with matched controls, even when the most restrictive thresholds are used. The lack of correlations among GH peak, parameters of fat distribution and low serum IGF-1 in HIV patients suggests that impaired GH secretion may be not related to fat redistribution. Anyhow, these results do not permit to establish definitively whether females with HARS and with impaired GH response to GHRH+Arg are truly or functionally GH deficient.

P448

Glucose infusion affects memory function but not ACTH concentrations in patients with Addison's disease

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Background
Sucrose intake has been shown to normalize the disturbed neuroendocrine stress response in adrenalectomized rats. These previous data indicate a compensatory effect of energy supply on altered parameters in chronic cortisol deficiency. Thus, we hypothesized that glucose infusion may have similar beneficial effects in patients with Addison's disease.
Methods
We examined 10 patients with primary adrenal insufficiency who discontinued their hydrocortisone substitution and 10 matched healthy controls in two randomized conditions. Subjects received either an intravenous glucose application (0.75 g glucose/kg for 2.5 h) or a sodium chloride infusion. We assessed parameters of cognitive function (short-term memory, selective attention) as well as symptom scores and measured concentrations of the relevant hormones in this context (ACTH, cortisol, catecholamines, GH, glucagon).
Results
In the Addison group, recall of emotional words was enhanced by glucose infusion, whereas recall of neutral words was impaired. Glucose infusion did not influence word recall in the control group. Selective attention and symptom scores were not affected by glucose infusion as compared to placebo, but selective attention was generally lower in patients than in controls under both conditions. Concentrations of ACTH, cortisol, GH, and glucagon showed no significant difference between conditions in both groups.
Conclusion
Our data do not confirm a compensating effect of glucose infusion on neuroendocrine parameters in chronic cortisol deficiency in humans. However, glucose affected short-term memory in a differential way with improvements in the emotional task while neutral memory was impaired. Since emotional memory is primarily amygdala-dependent while neutral memory processing is rather assigned to the hippocampus, one could speculate that glucose application may cause differential effects on these brain areas in patients with Addison's disease.

P449

Prevalence of metabolic syndrome in adult hypopituitaric patients with GH deficiency (GHD)

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The prevalence of the metabolic syndrome is increasing worldwide. The untreated metabolic syndrome places individuals at risk both for diabetes and cardiovascular disease. Adult GH deficiency syndrome present some of the 5 features of the metabolic syndrome, abdominal obesity, high triglycerides, low HDL-cholesterol, elevations of blood glucose, hypertension and a 2-fold higher

risk of death for CD compared with controls. The aim of this study was to determine the prevalence of metabolic syndrome among adult hypopituitaric patients with GHD. Follow-up was undertaken of 100 GHD hypopituitaric adult patients. Testing included fasting insulin, glucose, lipids and GHRH + ARG test and IGF-I. The results were compared with population norms from 1990–2000 of Osservatorio Epidemiologico Cardiovascolare (OEC) data. The patient and control age range was 35–74 years. The prevalence of metabolic syndrome was significantly higher in patients than in population controls (35% vs. 23%, $\chi^2 = 7.35$; $P = 0.007$). In particular hypertension was found in 41% of patients and in 32% of controls ($P < 0.05$), hyperglycaemia in 14% of patients and 7.5% of controls ($P = 0.02$) and hypertrygliceridemia in 52% of patients and 23.6% of controls ($P = 0.000$).
In conclusion, adult GHD hypopituitaryc patients have a higher prevalence of metabolic syndrome when compared to normal populations. Thus, these patients should be carefully monitored for cardiovascular and diabetes risk profiles. Whether GH replacement therapy could reduce this risk remains to be established.

P450

The benefit of long-term growth hormone replacement therapy in adults, results of the German KIMS database

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Objective
The German KIMS Database is a national surveillance study for evaluation of efficacy and safety of growth hormone (GH) replacement therapy in adults with GH deficiency (GHD) in clinical practice.
Patients
The analysis was performed using data of 1425 consecutively documented adult patients (777 men, 648 women) with GHD enrolled in KIMS Germany. The present report examined baseline and long term data (>48 months, range: 48–161 month) from a subset of 780 of these patients (420 men, 360 women) with GH deficiency aged 20–75 (median 44) years. Patients have been assigned to sex and age-related groups (20–39, 40–59 and 60–79 years). Patients were examined for serum IGF-1, blood glucose and lipid profile at baseline and at last visit. Furthermore QoL-AGHDA score has been determined for quality of live assessment. Most of the patients required additional pituitary replacement and were on optimal doses at recruitment.
Results
The IGF-1 and IGF-1 SDS-levels increased in all groups significantly ($P < 0.001$). After GH therapy serum total cholesterol and low-density lipoprotein (LDL) decreased in males (Δ −0.2 mg/dl, $P < 0.005$) and females (Δ −0.2 mg/dl, $P < 0.005$) significantly. Blood glucose increased from 4.7 to 4.9 mmol/l ($P < 0.01$) and in 15 patients the development of diabetes has been observed. The QoL-AGHDA score improved from 8 to 3 ($P < 0.001$) in all groups significantly. Moreover we detected no influence of seasons on quality of live assessment. During the treatment recurrences of pituitary tumors $n = 44$ (5.6%) or further neoplasia $n = 23$ (2.9%) have been observed.
Conclusion
Therefore, these observational data showed significant long-term efficacy of adult GH replacement therapy on IGF-1, lipid profile as well as quality of life.

P451

Panhypopituitarism due to hemochromatosis: a case report

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Introduction
Hemochromatosis is an iron storage disease characterized by iron deposition in parenchymal cells due to increased intestinal iron absorption. Iron overload leads to tissue damage and dysfunction particularly in the liver, pancreas, heart, joints, and pituitary gland. Panhypopituitarism is a clinical condition in which the anterior pituitary gland hormones are deficient. Herein we report a very rare case of panhypopituitarism due to hemochromatosis.
Case
Sixty-two year old woman was admitted to a medical center because of hematemesis 6 years ago. An upper gastrointestinal Endoscopy revealed the presence of esophageal varices. Laboratory findings showed positive antibodies to HCV. 1.5 years ago performed liver biopsy established the presence of micronodular cirrhosis with accumulation of hemosiderin. Laboratory findings were as follows iron binding capacity: 120 μg/dl; Fe: 127 μg/dl; Ferritin: 581 ng/ml. Abdominal ultrasonography showed chronic liver parenchymal disease, splenomegaly (15.5 cm) and cholelitiazis. She hospitalized to the intensive care unit with the diagnosis of chronical liver parenchymal disease and hepatic encephalopathy, and therapy started. Her thyroidal function tests were as follows f-T4: 0.396 pg/ml, f-T3: <1 pg/ml, TSH: 0.795 IU/ml. We analysed other hypophysary hormon tests because we thought hypophysary involvement due to hemochromatosis. The values were LH: 1.12 IU/ml, FSH: 2.77 IU/ml, GH: <0.05 ng/ml, prolactin: 0.657 ng/ml, cortisol: 3.54 μg/dl. We performed ACTH stimulation test for hypophysary insufficiency (Synacten 1 mg IV). In 0 min cortisol level was 3.54 μg/dl, 30 min 20 μg/dl and 1 h 10 μg/dl. Therefore, we excluded primary adrenal insufficiency. The contrast hypophysary computerized tomography was normal. So we diagnosed the patient as panhypopituitarism. We administered prednisolon 40 mg initially and levothyroxin on the fifth day. She recovered with this therapy and discharged with prednisolone 7.5 mg/d. and levothyroxin 0.1 mg/d.
Discussion
Hemochromatosis may rarely cause panhypopituitarism by accumulation of hemosiderin. The clinician must be careful when a problem about conscious occurs in patients with cirrhosis due to hemochromatosis. Hepatic encephalopathy may confuse with panhypopituitarism. The differential diagnosis should be made otherwise the treatment might be delayed. In conclusion, the patients with hemochromatosis who have cirrhosis should be assessed about pituitary involvement.

P452

Pituitary autoantibodies in patients with central diabetes insipidus and polydipsia: evidence of autoimmune pituitary involvement in both states

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A role of autoimmune aggression is postulated in the pathogenesis of different diseases in endocrinology. Forty to sixty percent of cases of central diabetes insipidus (CDI) are considered to be idiopathic. Primary polydipsy is a diagnosis of an exclusion of diabetes insipidus on the basis of water deprivation test, but sometimes it precedes the overt CDI. Thus the aim of this study was to define the prevalence of pituitary autoantibodies in patients with CDI and primary polydipsia.
We studied serum samples from 110 individuals: 61 – with CDI, 16 – with primary polydipsia, as defined by water deprivation test and 32 from control group matched by sex and age. Among patients with CDI: 36 had idiopathic form of the disease, 10 – postoperative, 8 – had tumors of the sellar region, 3 – head trauma, 2 – postinfectious, and 2 – due to hystiocytosis X.
We used solid-phase immunoenzyme assay and human hypophyses for evaluation of autoantibodies (membrane and cytosol fraction). Results showed high prevalence of pituitary autoantibodies in the group of CDI (50.8%) In subgroups being: idiopathic – 61.1% (22), postoperative – 20% (4), tumors – 25% (2), trauma – 2 patients, hystiocytosis – 1 patient, postinfectious – none. Prevalence of pituitary autoantibodies in group of polydipsia was 37.5% (6) Control group revealed 9.3% (3).
Data shown confirms the endowment of autoimmunity in development CDI with its significant role in idiopathic form of the disease. Our results also indicate surprisingly high prevalence of pituitary autoantibodies in group of patients with primary polydipsia which may represent the early phase of autoimmune CDI.

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Endocrinopathies with langerhans cell histiocytosis (LCH): nine cases

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LCH is a rare proliferative histiocytic disorder, and it can infiltrate virtually any site in the body. Diabetes incipidus (DI) is the most common abnormality when there is involvement of the hypothalamic-pituitary axis (HPA). We have evaluated the anterior pituitary function and their responses to treatment in 9 patients (5M/4F; range 19–60 years) with proven LCH and DI. Endocrine evaluations consisted of clinical history, basal (GH, IGF-1, fT4, TSH, PRL, cortisol, LH, FSH, E2, and testosteron levels) and dynamic pituitary function tests, plasma and urine osmolalities/water deprivation test at the time of diagnosis of DI and thereafter. The mean age at onset of DI was 28 years (15–60). Radiological evaluation included MRI of the HPA region. Three patients with infundibular and one patient with thalamic involvement were treated with local radiotherapy (RT). Chemotherapy and local (bone) RT combination had been given to 3 patients with bone lesions, and another 2 had been treated with chemotherapy alone. Median follow-up period was 74 month (2–300). On admission, single-organ involvement in one of the patients, and multisystem involvement in the remaining 6 patients were found. In addition to DI, diagnosis of secondary hypogonadism was established in only one patient who had no signs of puberty but normal PRL level. Another one had also secondary hypothyroidism, hypocortisolism, and hypogonadism with thalamic involvement. The other 2 patients did not suffer from any additional abnormalities concomittant with the initial DI. Hyperprolactinemia was found in 3 patients (43%). MRIs showed infundibular enlargement (89%), thalamic mass (11%), and the absence of the bright spot (100%) on the T1-weighted sequences. One patient had an additional mass in the pons with partially empty sella. Two patients who had received local RT to the pituitary stalk achieved complete radiological responses, and gonadotropin deficiency also recovered in one of them. One patient with pons and another with thalamic involvement died after therapy. Until now, GH deficiency developed in 5 patients and partial gonadotrophin deficiency in another patient. Seven patients are currently under regular follow-up with stable disease, but DI persisted in all patients. As a result, DI in LCH was the earliest hormonal deficiency. Localized RT can be successfully applied as a single treatment for infundibuler involvement or in combination with chemotherapy leads to high remission and local control rates.

P454

Aryl hydrocarbon receptor interacting protein (AIP) expression in human pituitary adenomas

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Background
Germline AIP mutations confer a predisposition to pituitary adenomas (PA), usually in the setting of familial isolated pituitary adenomas (FIPA); AIP mutations account for 50% of familial acromegaly. AIP-related PA are GH, PRL-secreting or non-secreting. Little is known about AIP expression in PA. Although the prevalence is low, identifying AIP mutations in apparently sporadic PA is important for studying at-risk relatives. Pre-screening criteria would help select patients for mutational analysis.
Material and methods
AIP expression was studied by Real Time RT-PCR in 45 PA, including 3 FIPA acromegaly cases with AIP mutations (AIP^{mut}), and by immunohistochemistry (IHC) in 56 PA, including 9 AIP^{mut} cases (7 GH, 1 PRL and 1 non-secreting PA). AIP immunostaining was scored semi-quantitatively. All PA phenotypes were represented and normal pituitaries (NP) were used as controls.
Results
AIP transcripts were detected in all cases. Mean transcript levels in PA were similar those observed in NP, but variations were observed, with 20% with moderate overexpression (mainly non-secreting and somatotropinomas) and 10% with underexpression, including the 3 AIP^{mut} cases. AIP protein was detected in most PA (49/56), with immunostaining generally less intense than in NP ($P<0.001$). The strongest immunostaining was observed in somatotropinomas, though AIP^{mut} somatotropinomas had a lower AIP score than other somatotropinomas ($P=0.023$). Only 2/9 PA from AIP^{mut} patients (1 GH-, 1-PRL-secreting) showed a complete loss of immunostaining.
Conclusion
AIP down-regulation is frequently observed in PA, mostly at a protein level. AIP^{mut} somatotropinomas show AIP down-regulation at both mRNA and protein levels, but AIP immunostaining is totally abolished in only a minority of cases. Though promising, further experience is needed to integrate IHC as a pre-screening tool for AIP mutational studies in patients with PA.

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Systemic comorbidities induce early vascular alterations in patients with active acromegaly

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Mortality risk is increased in acromegalics, due to cerebrovascular and cardiovascular events. These events are namely related to atherosclerotic vascular alterations, both at coronary and peripheral level. Systemic comorbidities increase the risk of atherosclerosis, but the role of GH and IGF-1 excess is still debated. To evaluate the relationship between GH/IGF-1 excess, systemic atherogenic complications and vascular alterations we investigated stiffness index (β), pulse wave velocity (PWV) and intima-media thickness (IMT) of right and left common carotids in 11 patients (9F:2M, mean age 52.8 ± 4.2) with active acromegaly complicated by systemic comorbidities (hyperlipidemia, blood hypertension and/or diabetes mellitus), in 8 healthy subject (HS, 6F:2M, mean age 48.8 ± 3.2) and in 11 non acromegalic patients matched for comorbidities (NP, 7F:4M, mean age 55.7 ± 3.3). To exclude the role of aging in atherosclerosis, patients and healthy subjects were younger than 60 years of age. Acromegalics showed higher β, PWV and IMT than HS (β: 9.2 ± 1.8 vs 5.3 ± 1.7, P 0.0004; PWV: 7.0 ± 0.8 vs 4.8 ± 1.0, P 0.0004; IMT: 0.9 ± 0.2 vs 0.4 ± 0.1, P 0.0001) but not than NP. The β (8.6 ± 1.3 vs 13.1 ± 1.4, P 0.005) and PWN (7.0 ± 1.0 vs 8.5 ± 0.5, P 0.005), but not IMT, were significantly increased in patients with longer duration of acromegaly (≤10 years vs >10 years). Serum GH and IGF-1 S.D. values did not correlated with β, PWV and IMT in acromegalics and no differences in these index were found on the basis of number of comorbidities (acromegalics with 1 comorbidity vs acromegalics with 2 or more comorbities). In conclusion, 40–60 years old acromegalic patients are at risk for early atherosclerotic vascular abnormalities more than healthy subjects and systemic comorbidities play a critical role on these alterations. Despite the increase of atherosclerotic index is not correlated with GH and/or IGF-1 concentrations, early vascular abnormalities are increased in patients with longer duration of the disease.

P456

Use of videocapsule endoscopy (VCE) for the detection of small bowel tumors in patients with acromegaly

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A high risk for small bowel (SB) tumors in acromegalic population has been reported in a cohort study (Baris D, 2002). SB lesions may be easily investigated by the recently developed videocapsule endoscopy (VCE). Aim of the study was to assess the prevalence of SB neoplasms by VCE in 14 acromegalic patients (Ac) in respect to 30 sex and age-matched control subjects and to correlate it with cancer risk factors and acromegaly-related parameters. Local Ethical committee approved the study. The Ac group (5M and 9F, age ± S.D.: 52 ± 11 years), included 4 patients cured by surgery and 10 medically treated (9 by somatostatin and 1 by dopamine agonists, 7 of whom with controlled disease). Cancer risk factors were similar in Ac and controls. History of the disease, GH and IGF-I levels, IGF-II and IGF-BP3 levels and metabolic parameters such as glucose tolerance, insulin resistance and insulin secretion were in addition investigated in Ac. Four gastrointestinal stromal nodular tumors (GIST) and 1 polyp of SB were detected in 16% of controls and in 29% of Ac (2 GISTs and 2 SB polyps, *P*: NS). In Ac the calculated relative risk for overall SB neoplasms was 1.74 (95% CI: 0.5418–5.424, *P*: NS), and for SB adenomas was 4.28 (95% CI: 0.4230–43.422, *P*: NS). Ac with positive VCE had a significantly longer duration of active disease (128.5 ± 107.5 vs 45.0 ± 44.6 months, $P<0.05$) than Ac with negative VCE, in spite of a younger age (48 ± 4 vs 54 ± 13 years, *P*: NS). No correlation with control of the disease, the presence of colon cancer and any other investigated parameters was found. In conclusions, these preliminary results show that acromegalic patients, mostly those with a long duration of active disease, might have a high risk also for SB tumours development, even if larger case studies are needed. VCE is a useful adjunctive diagnostic tool in acromegaly.

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Fatigue in breast cancer patients during chemotherapy: correlation with neuromuscular dysfunction and IGF-1 plasma levels

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Introduction
Adult growth hormone deficiency is associated with fatigue, tiredness and myalgias. The same clinical pattern can often be present in oncological patients during chemotherapy and follow up.
Aim of the study
To conduct an extensive neuromuscular investigation of patients with breast cancer (BC), treated with taxol, in an attempt to explain their neuromuscular symptoms, eleven pre-menopausal patients with BC underwent a prospective protocol, including hormonal examination and a neurophysiological study that comprised electromyogram (EMG) of sural nerve. All patients were even evaluated for IGF-I plasma levels at the time of diagnosis of BC and during follow up.
Results
Sensory neuromuscular examination showed a progressive derangement of EMG suggestive for a neurogenic demage. A reduction of IGF-1 plasma levels was observed from baseline to follow up ($P<0.05$) where a direct positive correlation with IGF-I plasma levels was also found ($P<0.05$).
Conclusion
The neurophysiological study confirmed the presence of sensory neuropathy of sural nerve that can be a cause of fatigue in our series of patients; the presence of low plasma levels of IGF-1 at follow up and a direct correlation of these data with EMG was suggestive for a possible direct involvement of somatotrope axis in explaining, in part, the presence of fatigue in breast cancer patients.

P458

Effect of GH replacement on coronary flow reserve (CFR) and on some cardiovascular risk factors in adult GHD patients

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GH deficiency (GHD) negatively influences cardiovascular function directly by impairing cardiac filling, performance, and contractility and indirectly by inducing atherosclerotic changes, hypercoagulability, abdominal obesity, insulin resistance, dislipidemia, endothelial dysfunction, etc. This accounts for the reduced life expectancy and increased risk of death for vascular disease in these patients.
CFR represents the capacity of the coronary circulation to dilate following an increase in myocardial metabolic demands and is considered an endothelial function index.
We investigated the cardiac and endothelial function and evaluated coronary microvascular circulation in a group of adult onset GHD patients during the first year of GH replacement therapy.
We studied 13 (7 males, 6 females aged 56.43 ± 4.49 years) patients (all nonsmokers, non diabetics, without hypertension or vascular disease) with adult-onset hypopituitarism and GHD before and after 1 year of GH therapy. In these patients CFR, echocardiographic analysis (cardiac mass, systolic and diastolic function), IGF-I plasma levels, lipid profile, HbA1c, blood pressure and anthropometric data were recorded before GH therapy and after 12 months of continuous therapy (mean starting dose 1.2 ± 0.19 mg/week adjusted every 8–10 weeks on the basis of IGF-I response).
After GH administration a significant improvement of cardiac mass ($P<0.02$) and CFR (from 2.35 ± 0.24 to 2.88 ± 0.25; $P<0.02$) has been observed; systolic blood pressure decreased significantly (from 135.38 ± 3.51 mmHg to 125.77 ± 4.04 mmHg; $P<0.02$) as well as LDL Cholesterol (from 143.25 ± 7.16 mg/dl to 124.51 ± 5.99 mg/dl; $P<0.05$). Improvement in diastolic filling was also observed.
In conclusion these data show that GH therapy improves endothelial and cardiac performance and, therefore, can prevent the progression of the atherosclerotic processes in GHD by reducing these cardiovascular risk factors.

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Effect of cholescytokinin (CCK) on food intake and brainstem and hypothalamic neuronal activation and its modulation by glucocorticoid

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CCK, secreted from duodenal cells in response to nutrients is involved in the satiety mechanisms, via activation of nucleus tractus solitarius (NTS). The arcuate (ARC) and paraventricular nuclei (PVN), which receives projections from NTS, integrate the circuitry that controls food intake. Corticotrophin releasing factor (CRF) participates in the energy homeostasis, decreasing food intake.
To evaluate the effect of glucocorticoid on feeding and neuronal activation after CCK treatment, Wistar rats were subjected to Sham surgery, adrenalectomy without (ADX) or with corticosterone replacement (ADX + B). Animals fasted (day 6) for 16 h received ip injection of CCK 3.5 μg/kg or vehicle for determination of food intake and CRF and Fos imunohistochemistry.
Lower food intake was observed in Sham and ADX + B rats after CCK, compared to the respective vehicle-treated groups. However, this hipophagic response induced by CCK was not observed in ADX rats. CCK increased Fos expression in the NTS in Sham, ADX and ADX + B animals, with no difference among the three CCK-treated groups. We observed a higher number of Fos imunorreactive neurons in the ARC and Fos/CRF neurons in the PVN in ADX group, compared to Sham group, both after vehicle and CCK ($P<0.05$). We demonstrated that ADX abolishes the hypophagic response induced by CCK, however, NTS neuronal activation was not altered by glucocorticoid deficiency.
These data suggest that in the absence of glucocorticoids, other factors besides CCK participate in the satiety mechanisms, leading to a decrease of food intake. The increased activation of hypothalamic anorexigenic pathways during fasting, may contribute to the lower food intake observed with glucocorticoid deficiency.

P460

Intrasellar and parasellar tumors – concomitant symptoms and clinical syndromes

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Introduction
Pituitary gland, sella turcica and parasellar region can be involved by wide variety of lesions. Diagnosis of this pathology demands a multidisciplinary effort, especially endocrinological, ophthalmologic and neurological procedures. Each of these entities has unique diagnostic and treatment considerations.
Aim
Review of clinical symptoms related to sellar–parasellar region tumors and documentation of heterogeneity of clinical syndromes accompanying their diagnosis in own material.
Material
105 pts (63F; 42M) aged 18–83, observed in 2000–2007 in our department.
Method
Analysis of clinical picture, hormonal and visual studies.
Results
Intrasellar pituitary adenomas composed 85% (89 pts) of the whole group, including 73 macroadenomas (with 1 Nelsons' syndrome and 3 corticothroph tumors) and 16 microadenomas (1 TSH-oma). Among intrasellar tumors of extrapituitary origin 2 chordomas and 1 meningioma have been found. Extrasellar tumors comprised 14.3% (15 pts) of cases, with 3 craniopharyngiomas, 2 meningiomas, 2 cysts, 1 germinoma and 3 extremely rare: involving the sella brown tumor, pituitary stalk granular cell tumor and located in sphenoid sinus somatotroph adenoma among them. In four remaining patients pituitary metastases of breast (2 pts), lung (1) and prostate (1) carcinomas have been recognized. In the group of hormonal active pituitary adenomas we found: acromegaly in 21 subjects, prolactinoma in 10, Cushing disease in 8 and secondary hyperthyroidism in 1 patient. Among pituitary adenomas 51.5% were hormonally inactive. Parasellar tumor invasion (cavernous sinus, suprasellar region, sphenoid sinus) was observed in 78.1%. At least one of the mass effects (headache, cranial nerves paralysis, epilepsy, deafness, visual defects, anosmia, hyperprolactinemia and diabetes insipidus) or pituitary anterior lobe insufficiency have been found in 40 pts. Clinically evident pituitary apoplexy has appeared in 9 subjects.
Conclusion
Abundant symptomatology and disturbed pituitary functions caused by sellar–parasellar tumors require broad knowledge of several allied medical domains.

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d3-growth hormone receptor polymorphism (d3-GHR) is associated with low BMI and better glucose metabolism in acromegaly

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Background
The d3-growth hormone receptor (GHR) polymorphism is a common variant characterized by genomic deletion of exon 3 (d3) of the GHR gene. It could be linked to a better growth response to GH, but the findings are controversial. Due to the lack of IGF-I feedback on the tumoral GH secretion, acromegaly seems to be a good model to study functional characteristics of this polymorphism. Aim of the study was to investigate possible influences of d3-GHR on GH and IGF-I relationship and on metabolic parameters in acromegaly.
Methods
A retrospective study was conducted on 76 acromegalic patients Genotype analysis was carried out on blood leukocyte DNA by multiplex PCR assay. Clinical, hormonal and biochemical parameters were considered at diagnosis and taken from medical records of the patients.
Results
Forty-one patients (54%) were homozygotes for the full length GHR (d3−), while 35 patients (d3+) carried one (fl/d3 GHR, $n=28$, 36.8%) or two (d3/d3, $n=7$, 9.2%) copies of the d3 allele variant. No significant differences in sex, age, tumor size, GH and IGF-I levels, fasting serum glucose and insulin levels were found between patients d3− and d3+. Overall a positive correlation between log GH and IGF-I levels was found, but the relationship was similar in patients carrying or not carrying copies of the d3 allele. Body mass index (BMI) was significantly lower in d3+ than in d3-patients (25.8 ± 2.1 vs 28.1 ± 4.8 kg/m^2, mean$\pm$S.D., $P=0.011$). In addition, serum glucose and insulin concentrations measured 2 h after OGTT in a subgroup of patients were also significantly lower in d3+. Finally, a linear regression analysis showed d3-GHR allele to be a significant negative predictor of HbA1c levels ($B=-0.8\pm0.3$, $P=0.025$).
Conclusions
This study supports the hypothesis that the d3 polymorphic variant of the GHR is functionally different from the full length variant mostly for the effects on glucose metabolism and body weight regulation.

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Correlation of IGF-1 and IGFBP-3 levels with liver function in patients following traumatic brain injury or subarachnoidal haemorrhage

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The prevalence of growth hormone deficiency (GHD) following traumatic brain injury (TBI) based on IGF-1 testing differs in literature between 10 and 40%. In our cohort the frequency of IGF-1 values <1 S.D. was 25%. If our patients had a minimal hepatocellular damage, the IGF-1 value could be <1 S.D. We evaluated the effects of BMI and liver function on IGF-1 and IGFBP-3 values in patients following TBI or subarachnoidal haemorrhage (SAH).
Fifty-eight consecutive patients (27 female, 29 men) of our centre (age 19–78 years; mean 49 years) following TBI ($n=33$) or SAH ($n=23$) underwent baseline testing for pituitary dysfunction including IGF-1. Liver function tests included serum bilirubin (bili), γ-glutamyl transpetidase (GGT), aspertat aminotransferase (GOT), alanine aminotransferase (GPT), high sensitive C-reactive protein (hsCRP), tumor necrosis factor (TNF) and retinol binding protein (RBP). The level of the main intravascular store of IGF-1, IGFBP-3 was assessed in a reference laboratory.
Eight of 58 patients showed IGF-1 <1 S.D. and 8/58 IGFBP-3 $<95\%$ reference range. The expected positive correlation of IGF-1 and IGFBP-3 ($P<0.0001$) was confirmed. HsCRP was significantly negative correlated to IGF-1 ($P=0.0173$) and IGFBP-3 ($P=0.0184$). Patients with elevated hsCRP levels showed significant lower values of IGF-1 and IGFBP-3. GGT was significantly negative correlated to IGF-1 ($P=0.0372$) and RBP was significantly positive correlated to IGFBP-3 ($P=0.0188$). Interestingly no correlation of the BMI with IGF-1 ($P=0.2152$) or IGFBP-3 ($P=0.3476$) was detected.
According to our results the liver function influenced secretion of IGF1 and IGFBP-3. Evaluations of IGF-1 levels <1 S.D. as baseline screening for GHD had to consider the liver function tests (GGT, RBP and hsCRP) irrespective of the BMI.

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Identification and molecular characterization of new somatostatin receptor subtype 5 truncated isoforms in rodents

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The neuropeptide somatostatin (SRIF) exerts a wide variety of actions through five SRIF receptors (sst1-5). However, not all SRIF actions can be explained by activation of the known sst. In this context, our research group has identified two novel isoforms of sst subtype 5 (sst5A) named sst5B and sst5C expressed in human and pig. These isoforms are generated by splicing of cryptic introns within the coding sequence, which alters the open reading frame, and results in new, truncated receptors with different size and sequence as compared to sst5A. Given that rodents are widely used to study the physiological importance of gene

products because of the ability to generate genetically modified mice over- or under-expressing the product of interest, the current study was focused on the identification of new murine sst5 isoforms. Use of molecular biology techniques to search for partial sequences of truncated sst5 isoforms in rodents led to the identification of one sequence in rat, and two in mouse, one of which showed high interspecific nucleotide and amino acid sequence identity. Conversely, these truncated murine sst5 were not homologues to the human and porcine sst5 isoforms identified originally. Undergoing studies indicate that these sst5 isoforms display differential tissue expression patterns. Use of mice models under different physiological conditions (i.e. fasting, obesity, SRIF-knockout) has revealed that these isoforms are also differentially regulated in both pituitary and hypothalamus. Taken together, the differential pattern of expression and regulation of the sst5 isoforms, coupled to the fact that these isoforms are highly conserved in rodents suggests that these new truncated receptors may be of physiological relevance. Future experiments will focus on the study of the potential involvement of these new sst isoforms in mediating the unique SRIF actions that can not be explained with the known sst identified to date.

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Posttraumatic hypopituitarism is associated with an unfavorable body composition and lipid profile, and decreased quality of life 12 months after injury

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Objective
To describe body-composition, lipid profile, and health-related quality of life (HRQL) in patients with traumatic brain injury (TBI) in relation to the development of posttraumatic hypopituitarism.
Design
Cross-sectional with a nested prospective sub-study.
Patients
The cross-sectional cohort included 104 hospitalized patients with TBI (26F/78M; age: median 41 years (range 18–64); BMI: 25 kg/m^2 (17–39); severity: mild (Glasgow Coma Scale score (GCS) 13–15) $n=44$, moderate (GCS 9–12) $n=20$, severe (GCS<9) $n=40$). A nested cohort of 46 patients was followed prospectively. The study was approved by the local Ethical Committee.
Measurements
Body mass index (BMI), waist circumference, lipid profile, total- and regional- fat mass was assessed 3 and 12 months (prospective) or only 12 months (cross-sectional) post-traumatically. HRQL questionnaires (NHP, EQ-5D and the GH deficiency specific instrument – QoL-AGHDA) were completed 'pre-traumatically', 3 and 12 months (prospective) or only 12 months (cross-sectional) post-traumatically.
Results
Patients with posttraumatic hypopituitarism had higher age-, gender- and BMI-adjusted 12 months LDL-cholesterol, waist circumference, and total fat mass ($P<0.05$ in all cases), and a higher increase in total cholesterol ($P=0.01$) during follow-up compared with sufficient patients. These findings were unrelated to 12 months IGF-I and IGF-I SD-scores.
Hypopituitary patients also had worse age-, BMI- and TBI severity- adjusted overall EQ-5D VAS ($P=0.03$) and QoL-AGHDA ($P=0.01$) scores, and worse NHP dimension scores of sleep ($P=0.03$), energy ($P=0.02$), and social isolation ($P=0.04$), compared to patients with an intact pituitary function.
Conclusion
Posttraumatic hypopituitarism was an independent predictor of the classical phenotypic features of hypopituitarism including an unfavorable lipid and body-composition profile, as well as worsened HRQL.

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Gh/Igf-1 control and tumor growth reduction in active acromegalic patients on Octreotide LAR

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Prospective non-comparable randomized clinical investigation of GH/IGH-1 control and tumor growth reduction in active acromegalic patients on Octreotide-LAR is presented. Fifty patients (pts) with newly diagnosed acromegaly (primary teratment, group 1; mean age 47.8±13.9 years old) and 39 pts after previous surgical and/or radiological treatment (secondary treatment, group 2; mean age 48.1±12.2 years old) were treated with Ocrteotide LAR 20–40 mg every 4 weeks, duration of treatment was 24 months.
Initial hormonal levels were [median (25%÷75%)]: group 1- GH 25 (12.9÷55) ng/ml; IGF-1 685 ng/ml (578÷843); group 2: GH 14 (6.6÷39.5) ng/ml; IGF-1 574 (443÷755) ng/ml. Clinical improvement and hormonal response (more then 30% decrease of the GH and/or IGF-1 levels) were observed in 44 (88%) pts from group 1 and 35 (95%) pts from group 2. Significant IGF-1 levels decrease without any changes of GH levels were observed in 3 (6%) pts from group 1 and 3 (7.7%) pts from group 2; markable GH levels decrease without changes of IGF-1 levels – in 7 (14%) pts from group 1 and 2 (5%) pts from group 2. Tachyphylaxis phenomenon was noted in 4 (8%) pts from group 1: both [GH+IGF-1] after 6–12 months of treatment in 3 pts, only IGF-1 after 3 months of treatment in 1 patient. In group 1 GH/IGF-1 levels after 12 months of treatment were 4.3 (1.9÷13.9) ng/ml and 453 (215÷568) ng/ml; after 24 months – 7.1 (1.3÷10.8) ng/ml and 469 (395÷670) ng/ml respectively. In group 2 GH/IGF-1 levels after 12 months of treatment were 3.7 (1.2÷5.1) and 220 (174÷330) ng/ml and after 24 months 5.8 (1.5÷10.2) and 328 (180÷476) ng/ml respectively.
Shrinkage of the pituitary tumor were observed in 58.4% of pts from group 1; degree of shrinkage was 13–99% from the initial tumor volume.
Thus, Octreotide LAR is an effective treatment of 88% of *de novo* patients with active acromegaly, and 95% of patients with previous surgical and/or radiological treatment. In our hogort of patients hormonal control was more effective in group with secondary medical therapy compared with newly diagnosed acromegalic patients. However, tumor shrinkage was more obvious in patients with primary medical treatment.

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Effects of growth hormone replacement in 28 patients with growth hormone deficiency

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Aim
To evaluate the response to growth hormone (GH) replacement in patients with growth hormone deficiency (GHD) after 1 year of treatment in terms of body composition, carbohydrate metabolism, lipid metabolism and quality of life.
Method and patients
Twenty-eight patients with GHD with the following characteristics: 16 males, 10 with radiotherapy and 18 with panhypopituitarism, were included in our study. Body mass index (BMI) and waist hip ratio (WHR) were determined as body composition parameters. The assessment of carbohydrate metabolism was made by measuring fasting plasma glucose and glycated hemoglobin. Total cholesterol, serum high density lipoprotein-cholesterol (HDL-c), serum low density lipoprotein-cholesterol (LDL-c) and triglycerides were evaluated as serum lipid markers. Quality of life was assessed by Nottinghan scale.
Results
The mean IGF-I level increased from 63.8±48.6 at baseline to 192.3±102.5 ng/ml, $P<0.001$. There were no significant changes in BMI and WHR (28.1±6.5 vs 28.1±7.0 kg/m^2 and 0.84±0.08 vs 0.84±0.06). Fasting plasma glucose and glycated hemoglobin did not alter significantly (86±10 vs 88±9 mg/dl and 5.3±0.4 vs 5.4±0.4%). All serum lipid markers experimented an improvement (total cholesterol, LDL-c and triglycerides decreased and HDL-c increased compared to baseline levels; 212±52 vs 202±39 mg/dl, 133±41 vs 126±28 mg/dl, 129±81 vs 126±60 mg/dl and 53±16 vs 54±20 mg/dl, respectively) but these differences were not statistically significant. Quality of life assessed by Nottinghan scale improved significantly (12±10 vs 8±8, $P<0.05$).
Conclusion
According to our data we can conclude that GH replacement in patients with GHD improves their quality of life and does not impair carbohydrate metabolism. Despite the data on literature, we have not proved a significant improvement on lipid profile.

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Starting dose of 10 mg Octreotide-LAR appears ineffective for biochemical control in the majority of acromegalic patients: interim analysis from the OASIS trial

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Octreotide LAR (SMS-LAR) is available in 10, 20 and 30 mg dosing. The relation-ship between SMS-LAR starting dose and GH, IGF-I and symptoms was exam-ined. The Observational Acromegaly Study on Impact of Sandostatin LAR (OASIS) collects data on GH, IGF-I, symptoms, safety and tolerability in recently diagnosed acromegalic patients. Data are collected under normal practice conditions over 12 mos. Eight hundred and sixty patients from 138 centers in 23 countries are enrolled; 353 patients have data available for analysis. One hundred and fifty patients (mean age 48 years, 75.3% with a macro-adenoma) started the study with SMS-LAR treatment. Eighty-three percentage started with 20 mg SMS-LAR, 10% ($n=15$ pts) with 10 mg, 7% with 30 mg and one patient with 40 mg. Patients with 30 mg starting dose had the highest GH and IGF-I levels at treatment start, patients with 10 mg had the lowest (Table). GH mean levels were significantly different for the 10 mg and 20 mg groups ($P<0.01$) and tended to be different for IGF-I ($P=0.06$). IGF-I levels were significantly different for the 20 mg and 30 mg groups ($P=0.01$). Twelve of the 10 mg starting dose patients had efficacy information at 3 month evaluation, of those 82% were not biochemically controlled. Only half of those uncontrolled were up-titrated to 20 mg. Prevalence of any symptoms at treat-ment start was highest in the 30 mg group (90%) followed by 20 mg (83.7%) and 10 mg (80%) ($P=0.88$). In conclusion, higher starting doses of SMS-LAR are asso-ciated with higher GH and IGF-I levels, suggesting that biochemical parameters, drive the choice of SMS-LAR starting dose. Starting dose of 10 mg appears ineffective for biochemical control in the majority of acromegalic patients.

SMS-LAR start dose (mgs)	Mean GH ± S.D. (ng/ml)	Mean IGF-I ± S.D. (ng/ml)
10	10.3 ± 9.5	583 ± 313
20	22.4 ± 37.0	800 ± 398
30	23.7 ± 20.2	1149 ± 525

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Factors associated with response to medical therapy in patients with Acromegaly

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Acromegaly is associated with increased morbidity and mortality. Surgery, radiotherapy (RT) and medical therapy are the treatment options to decrease GH and IGF-I concentrations to levels associated with cure or normalisation of mortality. We examined the response to dopamine agonists (DA) and somatostatin analogues (SSA) in 276 patients with acromegaly who received medical therapy during follow up (198 DA, 143 SSA). One hundred and seventy two had surgery and 73 RT prior to medical therapy. GH and IGF-I values before and 12 months after initiation of therapy were analysed. In the DA group basal prolactin levels did not predict response to therapy (median GH% reduction): hyperprolactinaemia 26.7% (10.4–48) vs normal prolactin 34.8% (0.2–53.2), $P=0.58$. Prior surgery was associated with a less marked GH% ($P=0.026$) and IGF-I% reduction ($P=0.0043$): surgery group 23.9% (−9.9–48.5) and 9.2% (−1.0–26.6), no surgery group 40.5% (15–71) and 40% (15.4–88.2). Prior RT was associated with an enhanced GH% reduction but no significant effect on IGF-I% reduction: no RT (GH 20.5% (−9.9–39.1), IGF-I 9.4% (−16.2–28.7)) vs RT (GH 50.8% (15.5–67.5, $P=0.0029$), IGF-I 22.2% (3.1–57.9 $P=0.07$)). In the SSA group there was no effect of prior surgery on %GH or IGF-I decrease, $P=0.63$ and 0.78, respectively. Prior RT did not have an effect on decrease in GH ($P=0.77$) but it lead to a lower IGF-I% reduction ($P=0.045$). The role of pituitary hormone deficiency in the response was assessed; no differences were found if there was ACTH or TSH deficiency. However in the DA group, gonadotrophin deficiency was associated with less marked decrease in IGF-I% (4.5 (−0.36–11.52) vs 25 (9.4–40.4), ($P=0.04$)). The efficacy of DA in patients with acromegaly is irrespective of basal prolactin levels. Prior surgery and radiotherapy are associated with differences in GH and IGF-I response to DA and SSA. However ACTH and TSH deficiency did not have an influence in the response.

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Diagnosis of SIAD: Fractional urate excretion closes the diagnostic gap in hyponatremic patients on diuretics

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Background
The syndrome of inappropriate antidiuresis (SIAD) is the most frequent cause of hyponatremia. Its diagnosis requires a decreased serum osmolality, urinary osmolality >200 mosm/kg, clinical euvolemia, and urinary sodium (UNa) >30 mmol/l. The natriuretic effect of diuretics impairs the diagnostic accuracy of UNa and thus, limits the diagnostic and therapeutic decision in hyponatremic patients substantially. We therefore examined the accuracy to predict SIAD of alternative markers, thought not to interact with diuretics.
Methods
In a prospective study 86 consecutive hyponatremic patients (serum sodium <130 mmol/l) were classified according to their history, clinical evaluation, and saline response (isotonic saline) into a SIAD and a non-SIAD group. In both groups the following biochemical parameters were tested in a diuretic collective (DC) and in a non-diuretic collective (NDC): UNa, serum uric acid concentration, fractional excretion of sodium, urea, and uric acid (FEUA). The parameters accuracy to predict SIAD was assessed with a receiver-operating-charcateristic curve analysis.
Results
31 patients were diagnosed SIAD (36%), 53 patients were classified non-SIAD (64%), the mean age was 66 ± 15 years, 57 patients were on diuretics (68%). In NDC, UNa was the most accurate test parameter to predict SIAD (AUC 0.96), however, showed a significant loss of accuracy in DC (AUC 0.80; $P<0.01$ vs NDC). In DC, FEUA had a significant better performance, compared with all of the alternative parameters (AUC 0.96; all $P<0.01$) and showed a positive predictive value of 100% choosing a cutoff value of 12%.
Conclusion
In patients not taking diuretics, UNa is a sufficient test parameter to predict SIAD. In patients on diuretics, FEUA is the most accurate predictor of SIAD and shows a discriminative quality, similar to UNa, in patients not taking diuretics.

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Circulating visfatin and adiponectin levels are reduced in women after long-term remission of Cushing's syndrome

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Metabolic syndrome and insulin resistance persist 5 years after remission of Cushing's syndrome (CS). Adiponectin and visfatin are two adipokines highly expressed in adipose tissue. Adiponectin is reduced in obesity and insulin-resistant states; visfatin has been shown in some studies to be reduced in obesity. The aim was to evaluate visfatin and adiponectin levels, body composition, insulin resistance and prevalence of metabolic syndrome in a cohort of women

with long-term remission of CS, and compare it with that of controls matched for sex, age and BMI.
Methods
We report 37 women in long-term remission of CS and 72 controls, in whom body composition was measured by dual-energy X-ray absorptiometry scanning. Circulating adiponectin, visfatin, insulin and lipid profile were measured. Insulin resistance was calculated using the formula of the homeostasis model assessment (HOMA). Metabolic syndrome was evaluated using the NCEP ATP III criteria.
Results
Patients had been cured of hypercortisolism for 11 ± 6 years and their current age was 50 ± 14 years. When compared with the controls, cured CS patients had more total fat mass (39.7 ± 7.4 vs 35.7 ± 6.5, $P < 0.05$) and trunk fat mass percentage (40.8 ± 9 vs 34.3 ± 8.3, $P < 0.05$) and less visfatin (15.57 (8.5–24.5) vs 19.9 (11.2–59.3) μg/ml, $P < 0.05$) and adiponectin levels (12.48 (5.02–32.4) vs 18.1 (4.5–56.5) μg/ml, $P < 0.05$). There were no differences in insulin, HOMA-IR and prevalence of metabolic syndrome (9%) between both groups. Visfatin negatively correlated with fat mass. No correlation between adiponectin and body composition were found.
Conclusions
Despite long-term control of hypercortisolism, patients who have suffered CS have a persistent increase in total and trunk fat mass and a reduction in circulating visfatin and adiponectin levels, which may contribute to their increased cardiovascular risk. These alterations are not related to metabolic syndrome or insulin resistance.

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The impact of conventional radiotherapy on Wisconsin card sorting test performance in acromegalic patients treated with transsphenoidal surgery

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Neurocognitive dysfunction has been described in patients following pituitary radiotherapy. However, the relative contributions of other variables such as hormone-deficiency states and surgery is still unknown. Our aim was to compare the results of an examination of executive function in acromegalic patients treated with transsphenoidal surgery (TS) alone to those obtained from patients treated with TS followed by conventional radiotherapy (CR) using a two-field technique. We retrospectively compared these two outcome groups and carried out a Wisconsin Card Sorting Test (WCST). Sixty-six patients, 26 men and 40 women, aged 55.2 ± 12.4 years, with an average duration of symptoms before diagnosis of 5.1 ± 3.7 years, were included in this study. Forty-two patients were treated only by TS and 24 received additional CR. There were no significant differences between groups in sex, age, average duration of symptoms before diagnosis, and mean GH and IGF-1 levels before TS (18.1 and 21.1 μg/l for GH, and 820.1 and 889 μg/l for IGF-1, respectively in both radiated and not radiated groups). Although there were more pituitary deficits in the radiated group when their executive functioning was assessed, the prevalence of growth hormone deficiency was similar in both groups, as well as the percentage of patients that achieved criteria for cure. The CR group performed significantly worse than the TS group, with mean centiles based on age-adjusted normative data of 10.5 vs 27.9 for perseverative answers ($P = 0.007$) and 11.7 vs 28.5, for perseverative errors in each group ($P = 0.012$). The CR group committed also more total errors and required more trials to achieve the first category but these differences were not statistically significant. In our study, postoperative CR in patients with acromegaly is associated with a poorer performance on the WCST when compared to TS alone, documenting specific problems in conceptual flexibility.

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Somatotropin release inhibiting factor (SRIF) downregulates Wnt/ß-catenin pathway in ACTH-secreting pituitary cell line AtT-20.

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Introduction
The involvement of the Wnt/ß-catenin pathway is apparent different epithelial human cancers. Wnt stabilizes ß-catenin (free ß-catenin) by preventing its phosphorylation which targets ß-catenin for degradation. Free ß-catenin is translocated to the nucleus where it stimulates a number of genes that modulate proliferation and differentiation upon binding to TCF/LEF-1. We have previously shown that Wnt/ß-catenin pathway is dysregulated in adrenocorticotropin (ACTH)-secreting pituitary adenomas. The therapeutic options for this tumor entity are limited. In this context, SRIF has been proposed as a therapeutic agent in the treatment of ACTH-secreting pituitary adenomas. The mechanisms remain largely unclear. The aim of the present study was to investigate the effect of SRIF on the Wnt/ß-catenin pathway in ACTH-secreting pituitary cells.
Methods
The ACTH secreting mouse pituitary cell line AtT-20 was treated with SRIF-14 and its effect on the expression of ß-catenin, GSK-3ß, TCF-4 and Cyclin D1 was analyzed both at the RNA and protein levels by RT-PCR and Western blotting, respectively. Additionally, the relative values of phospho-ß-catenin to total ß-catenin and phospho-GSK-3ß to total GSK-3ß were studied under the effects of SRIF-14 and forskolin (activator of cyclic AMP).
Results
In the ACTH-secreting pituitary cell line AtT-20 treatment with SRIF-14 rapidly decreased ß-catenin, TCF-4 and cyclin D1 mRNA expression at 4–6 h and caused a potent and long-lasting decrease also at the protein level. The downregulation of ß-catenin was blocked by forskolin. Furthermore, the phospho-ß-catenin/total ß-catenin ratio was higher in SRIF treated cells as compared to untreated controls and also to forskolin stimulated cells. These findings correlated well with the values of phospho-GSK-3ß, an inactive form of GSK-3ß, which was higher in untreated controls and forskolin treated cells comparative to SRIF treated cells.
Conclusions
Our data indicate that SRIF downregulates the Wnt/ß-catenin pathway and this effect can be blocked by the activation of adenylate-cyclase pathway in ACTH-secreting pituitary cell line AtT-20. Inhibition of Wnt/ß-catenin pathway by SRIF or its analogs might exert beneficial effects on corticotrope dysfunction in Cushing's disease.

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Identification of ACTH-secreting adenomas in Cushing's disease using intraoperative high-frequency direct contact ultrasound. Preleminary results

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Introduction
Up to 40% of cases with endocrinologically and surgically proven Cushing's disease (CD) are MRI-negative[1]. With intraoperative transsphenoidal ultrasound 72% of microadenomas in CD were identified as hyperechogenic structure[2]. We report on the first 9 cases with intraoperative use of direct contact high-frequency ultrasound (hf-us) in patients with CD.
Patients
All 9 cases (all female, age 31–71 years) revealed typical symptoms of CD, 2 were recurrencies.
Technique
During direct transnasal microsurgical operations the sellar compartment was investigated in axial and sagittal direction at 12 and 13 MHz through the pituitary capsule after drilling of the bony floor by use of a digital ultrasound probe (B-mode frequency 7.5–13 MHz, wide of field 5 mm, penetration 20 mm).
Results
In all 4 cases with negative preoperative MRI intraoperative hf-us correctly localized micoadenomas. In 2 out of 5 cases with positive MRI hf-us identified a hyperechogenic structure at the site expected (positive control). In 2 other cases with micoadenomas MRI correctly predicted the site of the tumor, but no identification was possible by hf-us. In the only case with a macroadenoma, identification of the border between tumor and anterior pituitary gland was not possible by use of hf-us. Out of 8 cases with microadenomas, in 6 the tumor was identified by hf-us (75%). Early postoperative decline of serum cortisol to subnormal levels on the first postoperative morning revealed surgically induced remission of hypercortisolism in all 9 cases[1].
Conclusion
Intraoperative, direct contact hf-us may enable the surgeon to identify small pituitary adenomas even in cases of negative preoperative MRI, thus preventing these patients from extensive pituitary exploration.
1. Knappe UJ & Lüdecke DK. *Neurosurgery* **39** 1996 484–493.
2. Ram Z *et al. JNS* **83** 1995 225–230.

P474

Macroprolactinaemia: a clinical problem

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Introduction
Prolactin is a hormone which is characterized with a high structural and functional polymorphism. Macroprolactin is one of the form of prolactin - it is a complex of monomeric prolactin and immunoglobulin. Macroprolactin is characterized with immunoreactivity and probably the lack of biological activity.
Purpose
The main purpose of our study was to estimate the frequency of macroprolactin appearance among the patients with hyperpronactinaemia but also analysis of clinical and biochemiacal symptoms of hyperprolactinaemia.
Material
The study involved 125 patients diagnosed of hyperprolactinaemia in term from January to December 2006.
Method
The concentration of total prolactin, macroprolactin (using the method of polyethylene glycol- PEG- precipitation) and recovery test of prolactin was marked to all patients. Macroprolactinaemia was diagnosed in case of recovery test below 40%. The frequency of clinical symptoms were evaluated in the patients.
Results
Among 125 patients with hyperprolactinaemia–macroprolactinaemia was diagnosed in 43 (34.4%).

Number of patients	Total PRL mIU/l	Free PRL mIU/ml	MRI	Clinical symp-toms	Oligo] menor-rhea	Galactor-rhea	Amenorrhea/ galactorrhea
43 (34.4%)	1658 657–5900	348 116–2514	Yes/no 35/8 2 tumors	11 (25.5%)	6	2	3

Among patients with clinical symptoms diagnosis of
– PCOS was established in 5 of 6 with oligomenorrhea (with free prolactin concentration- 245,142, 163, 157, 207 mIU/l respectively)
– hypergonadotropic hypogonadism in 1 of 6 with oligomenorrhea (free PRL-260)
– selective galactorrhea in 2 patients with galactorrhea (free PRL- 597, 643)
– hipogonadotropic hypogonadism in 3 patients with galactorrhea–amenorrhea with free PRL concentration of 2514, 1062, 928 mIU/l respectively.
Conclusion
1. Among patients with hyperprolactinaemia macroprolactinaemia is accounted for 34.4%.
2. Clinical symptoms do not appear in case of macroprolactinaemia.
3. Irregular menstruations and galactorrhea are results of coincidence of monomeric hyperprolactinaemia or other endocrine disorders (PCOS, premature ovarian failure)
4. Macroprolactin level measurement should be taken in every case of hyperprolactinaemia.

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Prolaktinoma and hyperprolactinemia in Albania

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Background
Prolactin hyper secretion is the most common endocrine abnormality due to hypothalamic–pituitary disorders. PRL is the hormone most commonly secreted in excess by pituitary adenomas.
Materials and methods
A retrospective study was performed during the period 1998–2007. The diagnose are based on objective examination, hormonal dosage, MRI.
Results
During these time were diagnosed 98 cases with hyperprolactinemia; 90.8% are females and 9.02% are males. Female/male ratio is 9.8:1.The mean age of diagnosis is 31.8 ± 10.5 years olds.
The diagnosis of a prolactinoma is confirmed by MRI: 35.8% of cases with microadenoma, 32.6% macroadenoma, 1% empty sella syndrome, 30.6% have normal MRI.
The mean value of PRL at the moment of diagnosis was 238 ± 257 ng/ml ($N = 1.9–25$).
Treatment
Prolactin microadenomas were treated with dopamine agonist drug. About 36.7% were treated with Bromocriptine and 40.8% with Cabergoline.
The mean PRL values before treatment with Cabergoline was 196 ± 16.1 ng/ml, after it 36.9 ± 53 ng/ml. The mean PRL values before treatment with Bromocriptine was 115.8 ± 10.8 ng/ml, after it 51 ± 66 ng/ml.
About 87.5% of macroadenomas underwent surgery due to the complication of pituitary mass. PRL values before surgery was 232 ± 154 ng/ml, after it 162 ± 168 ng/ml.
Because of the still high values of PRL after surgery, drug therapy was used in 59% of them.
Conclusion
The high percentage of macroadenomas is due to the late diagnosis of the cases. All the affected men present pituitary macroadenomas. Drug therapy is more effective than surgery alternative, but treatment should be prolonged.

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Prospective comparison of the glucagon stimulation test (GST) with the insulin tolerance test (ITT) in patients following pituitary surgery

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Objective
The ITT is the gold-standard for assessment of GH and ACTH reserve but has certain contraindications. GH and cortisol responses of < 3 ng/ml and < 500 nmol/l, respectively, have been defined as evidence of severe deficiency. The GST like the ITT stimulates both the ACTH and GH secretion and is suggested to be a good alternative in terms of efficiacy. However, there are limited prospective data with modern assays on sensitivity and specificity for the GST in comparison to the ITT. Aim of this study was an evaluation of the diagnostic utility of the GST in patients with hypothalamo–hypopituitary disease following pituitary surgery.
Design and patients
ITT and GST were performed within 7 days in 22 patients (14 men, age 28–61) at least 3 months after transsphenoidal surgery. Serum GH and cortisol were measured by Immulite 2000 assay (Siemens AG). ROC analysis was performed to identify optimal thresholds for GST; for cortisol deficiency analysis was adjusted to achieve a sensitivity $\geq 95\%$.
Results
Regarding GHD, 11/22 cases were classified as insufficient by ITT. For GST, ROC analysis revealed a cut-off of 3.1 ng/ml with 100% sensitivity and 81.9% specificity. Only 2/22 (9%) cases showed conflicting results compared to ITT in relation to this cut-off and were discordant in terms of defining GHD. Regarding cortisol deficiency, 6/22 cases were classified by ITT as cortisol insufficient. For GST, ROC analysis revealed a cut-off of 654 nmol/l with 100% sensitivity and poor 33.3% specificity. Using this cut-off, 10/22 (45%) cases showed conflicting results compared to ITT and were discordant in defining cortisol deficiency.
Conclusion
In our prospective series of patients with pituitary disease, the GST is a good alternative test for assessment of GH reserve, but poor for ACTH reserve, as demonstrated by comparison with the ITT. Test-specific cut-offs should be applied to avoid misinterpretation.

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Expression of ghrelin and opioid mRNAs in the hypothalamo–pituitary–adrenal axis after immune system activation

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The activity of the hypothalamo–pituitary–adrenal axis (HPA) is under control of endocrine, nervous and immune systems, in which opioids and recently

discovered ghrelin play important role. Mannan polysaccharide is often used as an alternatives to antibiotics, however, its role in the stimulation of the immune system is still uncertain. As a part of study dealing with the interaction of different factors during the growth and development the experiment was carried out in order to estimate the effect of prolonged treatment of mannan on the HPA synthesis of proghrelin and proenkephalin. Animal study were performed on the 30-days-old female lambs fed with standard food or with addition of prebiotic – mannan polysaccharide, isolated from the cell wall of Saccharomyces cerevisiae. During 30 days of experiment lambs were i.v. injected with saline (control) or naltrexone (3 mg/kg b.w.) five times every 7 days. Fragments of hypothalami, pituitaries and adrenals were taken out, frozen and directed to measurement of the expression of mRNAs for proghrelin and proenkephalin by the *in situ* hybridization method. Prolonged treatment with mannan significantly affected the expression of mRNAs for proghrelin and proenkephalin in the hypothalamus and pituitary. Unexpectedly, naltrexone, an opioid receptor antagonist, decreased the expression of mRNA coding for proenkephalin and increased for proghrelin in mannan fed animals.
In contrast, the response of both mRNAs in adrenals to polysaccharide treatment was very weak. Thus, the obtained results showed the interaction of hypothalamo–pituitary–adrenal opioids and ghrelin in the response to activated immune system.

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Cyclical Cushing's syndrome: prevalence in patients with Cushing's disease

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Background
Cyclical Cushing's syndrome has been considered to be a rare clinical entity, characterised by periodic increases in cortisol levels followed by regression of the Cushing's syndrome. The cycles of hypercortisolism may occur before the establishment of the diagnosis, rendering actual diagnosis difficult, or may occur after inadequate or ineffective treatment and affect disease management. The aim of this study was to investigate the prevalence of cyclicity of clinical symptoms and signs in patients with Cushing's disease, and to identify the characteristic features of this idiosyncratic population.
Methods
Two hundred and two patients with biochemically confirmed Cushing's disease, admitted from 1946 until 2001, were subjected to a retrospective case-note study. Cyclical disease was defined by the presence 1) intermittent signs and symptoms of Cushing's syndrome with or without biochemical confirmation prior to presentation; and 2) variability in signs, symptoms and biochemical findings during their follow up.
Results
Fifty-eight (24%) patients had evidence of cyclical disease. The patients were diagnosed at a mean age of 37.5 (median: 36; range: 7–95) years and were followed-up for a mean period of 14.5 (median: 14; range: 0–52) years. Evidence of cyclic disease before diagnosis was present in 28 (45%) and after diagnosis in 32 (55%) of patients. Before diagnosis the first evidence of cyclicity was present at a mean period of 5.58 (median: 5; range: 0–23) years before the established diagnosis. In the female population cyclical disease was present in 27% (41/154) and in the male population in 35% (17/48). About 16 (8%) patients were under 18 years old at presentation and cyclic disease was present in 2 (12.5%).
Conclusions
The findings in this large population study reveal that cyclic disease is not a rare presentation of Cushing's disease, and the physician should be alert to this possibility in making the diagnosis as well as after treatment in the follow-up period.

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Familial acromegaly: family screening and assessment in the familial isolated pituitary adenoma (FIPA)

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Familial acromegaly (FA) is a rare disease with less than 150 cases published. For its diagnosis (FA), two or more cases of acromegaly in the same family and the absence of MEN1 and/or Carney syndrome are required. FA is in the familial isolated pituitary adenomas (FIPA) group although its genetic condition is still under investigation.
The index case is an asymptomatic 43-year-old woman with a 4mm pituitary micro-adenoma. There were not acromegaly signs/symptoms, IGF-1 849.84 ng/ml and a glucose tolerance test with GH values: 21.4, 6.97, 2.11, 0.96, 0.47 ng/ml. Other hypophysis functions were normal. Genetic study for MEN1 was negative. She was operated in 2004 with an inmuno-hystologic study positive for GH, ACTH y PRL. She is still disease-free at the present time. Her father was diagnosed of a pituitary GH-secreting macro-adenoma (20×22 mm) in 1995. He had acromegalic phenotypic changes, basal GH: 20 ng/ml and post-glucose load GH level, 21 ng/ml. He died of a cerebrovascular accident before other diagnostic or therapeutic interventions were performed. Index case took part in the FIPA International Multicentre Study. The aryl hydrocarbon receptor interacting protein (AIP) gen mutations were negative for the more than 10 described mutations (also negative in 50% of FA and 85% in FIPA). It was also negative for the other 34 relatives (1st, 2nd, 3rd and 4th degree relatives in four different generations). All signed the informed consent form. A family tree was done of the three previous generations and, demographic and anthropometric data, GH and IGF-1 levels and a clinical symptoms questionnaire were collected. Several asymptomatic relatives with abnormal basal GH and IGF-1 values or height above 97% CI are still in the study. We describe a new family that meets the criteria of familial acromegaly in the FIPA group. FIPA would reach in the future a new clinical entity in the endocrine tumours classification. However, a more characteristic genetic pattern needs to be identified to be used as screening tool in potential FA.

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Hypopituitarism following traumatism brain injury in the West Indies: a pilot study between July 2005 and July 2006

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Sixty patients with traumatic brain injury are newly diagnosed every year in our island, particularly in patients with addictive behaviours. In France, it is the 4th rank of health expenses, as a major issue of public health care. In order to perform a first endocrine assessment, we performed a pilot study to diagnose traumatic brain injury induced hypopituitarism, assessed at least 6 months after injury, in moderate to severe traumatic brain injuries patients, hospitalized in our institution, from July 2005 to July 2006. It was a transversal study, approved by the Ethics Committee System of Bordeaux. All patients gave their informed consent after oral and written information. Over 65 medical files, 36 met our criteria: 12 patients died (6 in intensive care unit and 6 after their discharge); 14 out of 24 survivors were excluded after the first appointment (7 patients declined; 7 were lost for follow up). Of the 10 remaining patients, 6 showed traumatic brain injury hypopituitarism: 3 isolated growth hormone deficiencies (2 severe, 1 partial), 2 adrenocorticotropic hormone deficiencies associated with 1 partial and 1 severe growth hormone deficiency and 1 isolated thyroid stimulating hormone deficiency. This study pointed out the different difficulties we also met. This pilot study will lead to a further multicentric long-term study, in order to perform statistical analysis and to compare our results to those of the literature.

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Hypopituitarism after traumatic brain injury and its possible relation with neurocognitive and psychiatric disturbances

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Introduction
Recent advances in medicine have allowed a considerably decrease in mortality after traumatic brain injury (TBI) with the consequent increment in the number of subjects with physical, psychological and cognitive sequelas which means an important worsening of quality of life as well as difficulties in social and labour integration. Considering that many of the symptoms classically attributed to the TBI (memory impairment, concentration impairment, fatigue, social isolation etc.) are also associated with pituitary hormones deficiency, especially growth hormone deficiency, we thought about the possibility of studying if the presence of GH deficiency could be related with psychological and neurocognitive sequelas in TBI patients, and if a hormonal replacement treatment could be an adequate complement in the rehabilitation process.
Material and methods
Eighty-three patients were studied, 65 men and 18 women, with a mean age of 43.9 ± 1.9 years; age at TBI of 38.7 ± 1.9 years and body mass index (BMI kg/m^2) of 27.1 ± 0.4. The severity of injury was assessed by the glasgow coma scale (GCS) score; 57 patients had suffered a mild TBI, 10 patients a moderate TBI and 14 patients a severe TBI. All patients underwent: 1) A Basal Hormonal Study: ACTH, fT3, fT4, TSH, IGF-1, FSH, LH, testosterone (male) and oestradiol (women), PRL and ADH. To assess GH secretion the GHRH + GHRP-6 test was performed. 2) Psychiatric and neurocognitive evaluation: cognitive function by using Rey-Osterrieth Complex Figure y Digit Letter Substitution Test, and presence of psycho-psychiatric symptomatology by means of Beck's Depression Index and SCL-90 questionnaire
Results
In our patients neurocognitive assessment revealed a significant correlation of peak GH levels after stimulation with spatial construction, planning ability ($P = 0.02$) and with visual short memory ($P = 0.003$). A significant correlation of GH peak with DLST scoring, which is a measure of attention, perceptual speed, motor speed, visual scanning and memory was also observed ($P < 0.0001$). Psycho-psychiatric evaluation revealed a significant correlation of peak GH levels with PSDI (positive symptom distress index; ($P < 0.0072$).
Conclusion
It is important to establish the neurocognitive an psychiatric aspects potentially affected by hormonal deficiencies, especially GH deficiency, to assess the efficacy of hormone replacement therapy on neurocognitive and psychiatric alterations in TBI patients.

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Endoscopic pituitary surgery as the primary treatment for acromegaly: a prospective study in a specialised centre

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The main aims of treatment of acromegaly are reversing symptoms and signs, removing the tumour, preventing disease recurrence, and improving survival. Increased mortality associated with acromegaly can be diminished if treatment is successful in reducing GH <2–2.5 ng/ml. We carried out a prospective study to assess whether recent advances in surgical technique (namely the endoscopic approach in a specialised centre encompassing specialist pituitary surgeons working in team with endocrinologists, pathologists, and neuroradiologists) could obtain remission and stable disease control in the majority of acromegalic patients. Between 1998 and 2007, 152 consecutive acromegalic patients (65 men; 48 microadenomas; median age, 46 years, range 13–78) underwent pituitary surgery. Of these, 26 (17%) were operated for a residual tumour, while the remainder (83%) had had no previous intervention for their pituitary adenoma. On the basis of tumour extension, the pituitary adenomas were classified into grade 0 (18.4%), grade I (9.6%), grade II (57.5%), grade III (13.2%), and grade IV (1.3%). Surgery led to partial hypopituitarism in 2.6% and permanent diabetes insipidus in 1.3%. Based on biochemical criteria evaluated 3–6 months after surgery (mean GH <2.5 ng/ml, nadir GH on 75-g OGTT <1 ng/ml, and IGF-I in the normal age- and sex-related range), the overall remission rate was 68%, despite the lack of a residual tumour on MRI in 76%. A partial surgical success was achieved in 21% of cases (clinically relevant improvement with or without a residual tumour). The remainder 11% showed an active disease with a residual tumour on MRI. Our prospective study has showed that recent technical advances in surgical approach to the sella coupled with expertise surgeons working in team can obtain remission of the disease in about two thirds of cases, even though 17% of patients in our series had had a previous intervention.

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mTOR inhibitors rapamycin and RAD001 (Everolimus) induce antiproliferative effects in GH3 cell line and human pituitary adenomas

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The effect of mTOR inhibitors on pituitary tumors is unknown. Akt overexpression was demonstrated in pituitary adenomas, which may render them sensitive to the antiproliferative effects of these drugs.
To evaluate the *in vitro* effects of mTOR inhibitor rapamycin, and its orally-bioavailable analog RAD001 on pituitary cells, GH3 cells, a mammosomatotroph rat pituitary tumor cell line, and human GH-secreting and non-functioning pituitary adenoma (NFPA) cells were used.
Treatment of GH3 cells, cultured GH-secreting adenomas and NFPAs with rapamycin or RAD001 induced a significant dose- and time-dependent inhibition of cell viability. The inhibition of GH3 cell viability involved G0/G1 cell cycle arrest associated with cyclin D3 suppression. Expression of phosphorylated-p70S6K in GH3 cells, GH-secreting adenoma and NFPA cells was significantly reduced by rapamycin and RAD001. mTOR phosphorylation was significantly decreased by rapamycin and RAD001 in GH3 cells, while Akt phosphorylation was unchanged.
Our results showed that mTOR inhibitors potently inhibit pituitary cell proliferation suggesting that mTOR inhibition may be a promising antiproliferative therapy for pituitary adenomas. This therapeutic manipulation may have beneficial effects particularly for patients harboring invasive pituitary tumors unresponsive to current treatments.

Obesity

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The influence of low calorie-high dietary fibres diet on the change of parameters of the metabolic syndrome

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Metabolic syndrome is risk factor for cardiovascular event. According recommendation of NCEP ATP III, the major component of metabolic syndrome is waist circumference up to 94 cm in male and 80 cm in female, and two of following disturbances: fasting glucose up to 5.6 mmol/l, HDL below 0.9 in male and 1.3 in female, triglycerides up to 1.7 mmol/l and BP up to 135/80 mmHg.
We have analyzed the influence of low calorie diet (1200–1500 kcal/day) with increased dietary fibers of 25–40 g/day and the macronutrients ratio: fats 22–23%, proteins 15–18% and carbohydrates 55–65%. 95 subjects divided into two groups took part in this investigation. Examined group was treated with recommended diet and control group was treated with standard low calorie diet with dietary fibers of 10–15 g/day, carbohydrates 35–45%, fats 20–30% and proteins 20–25%.
After 6 months waist circumference decrease significantly ($P = 0.001$) in examined group, and non significantly increased in control group ($P = 0.17$). Fasting glucose decreased high significantly in examined group, and there was no changes in control group. HDL in examined group significantly increased ($P < 0.05$) while in control group there was no changes ($P > 0.05$). Triglycerides and systolic blood pressure decreases significantly in examined group ($P = 0.001$), but control group had more significant decrease of diastolic blood pressure ($P < 0.05$).
These results suggest positive effect of low calorie diet on the parameters of the metabolic syndrome. The reductions of total and saturated fats, balanced protein and carbohydrates intake and increased intake of dietary fibers have positive influence on the lipid profile and improve glycemic regulation in obese people with metabolic syndrome.

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Estradiol reverts the impairment of the natriuretic peptide system in a menopause-associated obesity model

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The follicle-stimulating hormone receptor knockout (FORKO) female mouse provides a useful model to examine the role that lack of estrogen plays in the development of obesity and hypertension in postmenopausal women. Estrogen increases circulating levels of atrial natriuretic peptide (ANP), a hormone with renal and cardiovascular effects. It has been shown that atrial natriuretic peptide (ANP) is a potent stimulator of fat cell lipolysis in addition of its well established effect in the blood pressure. The aim of this study was to determine the status of natriuretic peptide system in female follitropin-receptor knockout mice (FORKO) that could be associated with obesity and hypertension observed in these mutants. FORKO and wild-type (WT) mice received daily injections of 17β-estradiol (E_2) or vehicle, for 4 days**.** In the 5th day, blood was collected for determination of plasma ANP levels and some tissues were removed for determination of ANP, natriuretic peptide receptor type-A (NPr-A) and type-C (NPr-C) gene expression by RT-PCR. FORKO mice, that are obese, have lower circulating ANP levels and atria ANP gene expression and higher renal and adipocyte NPr-C gene expression than WT. E_2 treatment induced a significant reduction of body weight and mesenteric adipose tissue weight only in FORKO. This reduction was accompanied by an increase of plasma ANP and atrial ANP gene expression in FORKO compared to WT. E_2 treatment also induced a decrease of renal and adipocyte NPr-C gene expression in FORKO. In summary, this study shows that FORKO have an impaired natriuretic peptide system and that E_2 treatment improved this condition by increasing atrial ANP synthesis as well as by decreasing ANP clearance receptors, resulting in enhancement of circulating ANP level. Thus, estrogen regulated ANP system could have multiple roles in modulating obesity and hypertension.

P486

The effect of various dyslipidemia management guidelines on the assessment of LDL-C goal attainment in population-based studies

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Drug therapies are effective for improving lipid profiles. However, a large proportion of patients and specifically high-risk patients appear unable to achieve the recommended goals for LDL-C level. Furthermore, the proportions of patients, who lack the targeted LDL-C goal, are inconsistent among different studies.

Objectives

To explore if application of different guidelines for the management of dyslipidemia can influence the proportion of LDL-C target level achievement in a population level.

Methods

To assess the attainment of LDL-C goal, data from a Canadian community-based (CCB) study (*Clin Invest Med* 2007 **30** E63–E69) were evaluated against three guidelines: the 2003 Canadian guideline for management of dyslipidemia, the NCEP-APT guideline and AFSSAPS guideline. These result were also compared with goal attainment observed in other studies, including CALIPSO (Canada), L-TAP (USA), EUROASPIRE (Europe), OLYMPIC (Greece), REALITY-PHARMO (The Netherlands), BKL-Thales (France).

Results

Using the Canadian guideline, only 73% of patients in CCB study achieved the goal for LDL-C level. About 96% of low risk and 62% of high risk patients achieved the LDL-C goal. Similar results were obtained when data from the CALIPSO study were used. Conversely, the CCB study demonstrated a significantly different attainment of LDL-C targets compared with the studies from Europe and the United States that used the NCEP-APT guideline or AFSSAPS guideline to set the LDL-C target levels.

Conclusion

Several factors may contribute to the observed inter- country differences in goal attainment, including different patient populations, different clinical practice guidelines and different time frame when the results were obtained.

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Bariatric surgery for patients whit morbid obesity: a cohort study

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Objectives

Analyze the evolution of body mass index (BMI) and percentage of excess weight loss (PEWL) after bariatric surgery; compare adjustable gastric banding (LABG) vs laparoscopic gastric bypass (LGB).

Methods

Cohort study. Inclusion criteria: all cases underwent laparoscopic bariatric surgery; $n=89$; 9 patients were excluded for incomplete follow up. Differences between the 2 groups were evaluated using the Student *t*-test; $P<0.05$ was considered significant.

Results

$n=80$ (35 LABG/45 LBG).

Table 1 Baseline characteristics.

	Sex (male/female)	Age (years)	Hospital stay (days)	BMI (kg/m^2)
LABG	8.6/91.4 (%)	40±12.7	6.3±3.2	43.7±6.6
LBG	28.9/71.1 (%)	41.7±10.7	16.4±11.4	50.8±7.9

Table 2 Evolution of BMI and percentage of excess weight loss. Stadistic results.

	N (LABG/LBG)		LABG	LBG	*P*
3 months	35/45	BMI (kg/m^2)	37.4±5.5	39.4±5.8	–
		PEWL	30.1±10.7	39.4±9.7	0.001
6 months	33/45	BMI (kg/m^2)	35.7±5.2	35.2±6.3	–
		PEWL	37.9±14.6	56.9±12.1	0.001
12 months	27/43	BMI (kg/m^2)	34.6±4.8	31.5±5.6	–
		PEWL	46.8±18.1	69±17.3	0.001
24 months	17/24	BMI (kg/m^2)	34.9±4.4	32.4±5.8	–
		PEWL	41.6±19.8	66.1±20.5	0.001

Conclusions

Bariatric surgery is a successful treatment to obtain an adecuate weight loss. The best results are observed during the first year. LBG had better results.

P488

Overweight and obesity in Iranian adolescents

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Introduction

The problem of increasing prevalence of overweight and obesity as a consequence of new life styles is worrying the scientists and health officers in less developed countries. In Iran that is experiencing an accelerated nutrition transition overweight has turned into a major public health problem.

Objective

To determine overweight and obesity prevalence in Iranian adults.

Material and methods

We have used data on 35 924 individuals (17 996 male) from the National Food Consumption Survey. This survey recruited 7158 households from urban and rural regions of all the 28 provinces of the country. Age was confirmed by observing the ID, weight and height were measured due to standard protocols and BMI was calculated. Pre-obesity was defined as BMI $\geq$25 and obesity as BMI $\geq$30. Overweight was defined as the sum of the pre-obesity and obesity.

Findings

The average BMI was 25.5 among women and 26.4 among men. The prevalence of overweight was %42.4 among men and %56.5 among women. Obesity prevalence was %10 among men and %24 among women ($P<0.001$). Prevalence of pre-obesity and obesity among rural individuals is higher than their urban counterparts ($P<0.001$).

Conclusion

Our data show that the prevalence of overweight and obesity in Iran especially among rural women can be considered as a public health problem. After conducting analytic studies to determine the determinants and risk factors of overweight in different social layers, proper and feasible action plans are needed to slow down the accelerated trend of obesity.

P489

Obesity and it's hormonal profile in saudi population

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Objective
To evaluate and to study obesity in Saudi female population represente by Makkah community. This study aims to investigate obesity, by detecting leptin concentration and to take other parameters like body mass index (BMI) and waist circumference (WC) in assessing obesity.
Methods
Two-hundred forty women ($n=240$) between the ages 18 and 65 participate in this study. Volunteers were divided into three groups. The first group was the normal or control group with (BMI) range from 18 to 29.9, the second group were the obese and characterise with (BMI) ≥ 30 and finally obese diabetic group with body mass index (BMI) ≥ 30 and suffer from diabetes mellitus. Items collected included height, weight, waist circumference and blood sample. Blood samples were later thawed and serum leptin levels were detected with ELISA.
Result
Leptin was measured in all the groups and their means found to be (8.4 ± 1.4) in normal, (56.3 ± 18.8) in obese and (42 ± 19.3) in diabetic obese group. Leptin levels were directly associated with BMI ($r=0.152$, $P=0.178$) in normal group, and strong positive correlation in obese group and diabetic obese as the follow: ($r=0.350$, $P=0.001$), ($r=0.355$, $P=0.001$). Also, leptin concentrations were positively correlated with WC in obese and diabetic obese.
Conclusion
Leptin concentrations were found to be high in both obese and diabetic obese group and showed a directly positive relation with BMI and waist circumference. Understanding that leptin hormone influencing appetite and body weight that cause obesity. However, serum leptin concentration changes in response to many features like fasting, hypertension, practice physical activity, smoking or follow special diet.

P490

Adiponectin induces CXCL8 in primary human hepatocytes via the adiponectin receptor 1

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Background
Adiponectin was suggested to exert hepatoprotective effects in animal models of tetrachlorcarbonate-, endotoxin- and alcohol-induced liver injury. Further, in patients with non-alcoholic fatty liver disease (NAFLD) low adiponectin levels were found to be associated with more severe steatosis and hepatic inflammation.
Methods
To investigate the molecular basis of the hepatoprotective effects of adiponectin primary human hepatocytes were incubated with recombinant adiponectin for 24 h. GeneChip analysis was performed and CXCL8 mRNA was found induced.
Results
Adiponectin upregulated CXCL8 mRNA and induced CXCL8 secretion in primary human hepatocytes. Elevated CXCL8 in the supernatants was measured as early as 3 h after the addition of adiponectin to the hepatocytes. Adiponectin receptor 1 and 2 are expressed in hepatocytes and knock-down of AdipoR1, but not AdipoR2, by siRNA abrogated adiponectin induced CXCL8 release. The p38 MAPK inhibitor SB203580 did not significantly diminish adiponectin-mediated release of CXCL8. Electrophoretic mobility shift assays (EMSAs) revealed that adiponectin activated NFκB in primary hepatocytes but not in HepG2 cells. The hepatocytic cell lines HepG2, Hep3B and PLC were also incubated with adiponectin but CXCL8 release was not significantly induced in these cell lines.
Conclusions
The current experiments revealed that adiponectin activated NFkappaB and induced CXCL8 in primary human hepatocytes whereas hepatocytic cell lines were resistant to the effects of adiponectin. So far the role of CXCL8 in hepatic injury is not clearly established. Whereas CXCL8-mediated recruitment of neutrophils may be harmful to the liver, CXCL8 was also described to protect the liver against galactosamine/endotoxin and T-cell mediated apoptosis.

P491

Iranian overweight children and adolescents: who are seeking weight loss treatment?

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Objective
This study described overweight/obese children and adolescents seeking weight loss treatment regarding their age, gender, severity of obesity and maternal education in Rasht City, northern Iran.
Design
A descriptive study on overweight children and adolescents setting: the main clinic of obesity management in Rasht, northern Iran.
Subjects
Data on 1465 overweight/obese children and adolescents aged 2–18 years engaged in weight loss program were analyzed in this study. These data included age, sex, weight, height; self reported parental weight and height, history of dieting, and mother's level of education.
Results
Overweight/obese girls engaged in weight loss program more than overweight/-obese boys (71.2% vs 28.8% $P< 0.0001$). Only 3.2% of the children were in age group 2–6 years. These data showed that 18.2% of the overweight/obese children and adolescents were from families with low maternal education and the remainders were from families with high maternal education. Mean excess body weight was not different across educational levels although the boys were heavier than girls at. These findings showed that the maternal body weight was related to the child's excess weight ($r=0.26$ $P<0.0001$).
Conclusion
These data suggest that parents of overweight/obese children and adolescents from low social level, boys and young children across all maternal educational levels should be warned against risk of their children's accelerated growth.

P492

Metabolic and hormonal patterns in men under the age 40 years with obesity and metabolic syndrome

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Objectives
To assess the androgens profile (total testosterone (TT),free testosterone (fT), SHBG, and DHEA-sulphate), cortisol, aldosterone, insulin, and leptin in patients with obesity (OB), metabolic syndrome (MS) and healthy men (HM).
Subjects and methods
Group 1: 26 men with obesity, age 30 ± 6 years. BMI 27–35 kg/m^2. Group 2: 34 men with MS, age 31 ± 6 yrs. BMI 30–36 kg/m^2. Group 3: 20 healthy men, age 28 ± 5 yrs, BMI 20–24 kg/m^2. All antropometrical parameters such WC,HC, and WC/HC ratio were determined. Additionaly we used MRT. All biochemical variables (glucose, spectrum of lipids) were measured by standard methods. The levels of TT, DHEA-S, SHBG, insulin and leptin were measured in serum the using validated direct immunoassay method. Aldosterone was measured by RIA.
Results
Significant association between antropometric, metabolic and hormonal parameters was found. There was a progressive decline of plasma TT with increasing obesity, particularly in men with MS. There were no differences in cortisol levels between the groups.

Indices (Me)	Ms	Obesity	Healthy
TT nmol/l	11.2	14.8	21.9
fT pmol/l	258	280	372
SHBG nmol/l	24	35	40
Insulin μIU/ml	14	7	4
Leptin ng/ml	26	14	5
DHEA-S nmol/l	2841	3925	4617
Aldosterone pmol/l	459	335	244

Conclusion
Multivariate regression analyses shows that many predictive factors of variability in plasma TT levels are directly or indirectly related to SHBG in patients with MS. If plasma TT levels were included in the classification of diagnostic ATP III criteria, it would improve identification of MS from 79 to 85% among patients with obesity. Changes in aldosterone levels go in parallel with increasing insulin

and decreasing testosterone levels. Possible mechanisms of the above associations will be discussed.

P493

Prevalence of overweight in comparison to wasting, among Iranian under 5 year-old children in urban and rural

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Introduction and aim
Beside protein–energy malnutrition among children, high prevalence of overweight is also a public health problem and a major nutritional challenge in most developing countries. The aim of this research was to determine and compare the prevalence of wasting, overweight and obesity among Iranian urban and rural under 5 year -old children.
Subjects and methods
This study was based on national comprehensive study on household food consumption pattern and nutritional status (2001–2003). Seven thousand and one hundred and fifty eight households in rural and urban areas were selected randomly. Weight and height of 2562 children ($<$ 5 years) were measured and recorded using the standard protocol. Wasting was defined based on NCHS/WHO cutoffs (weight for age $\leq$2SD *Z*-scores, respectively) and overweight defined as weight for age $\geq$2SD. Data were analyzed using MS Access, and SPSS 11.0 softwares.
Results
Children under five years old were 7% of total population. The Prevalence of wasting among children was 10% (2–26%). The Prevalence in rural areas was 4% higher than urban areas (14% vs 10%, $P<0.01$).The prevalence of overweight were 3% (1–6%) and in urban and rural areas were similar (4% vs 4.5%).
Discussion
Evidence suggests that the prevalence of wasting among Iranian children especially among rural area is a major nutritional problem.
According to high prevalence of overweight and obesity among this age group, there is a need to implement nutritional strategies at micro and macro levels for preventing and controlling malnutrition.

P494

Gene expression profiling in skeletal muscle from diet-induced obesity susceptible rats

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Background
The variablity of the individual susceptibility to diet-induced obesity and insulin resistance is well known, but so far insufficiently explained. As skeletal muscle metabolism and insulin sensitivity are important pathophysiologic features of obesity-asscociated metabolic perturbations, we tried to identify differences in muscular gene expression profiles between high fat fed rats prone to develop obesity (diet-induced obesity, DIO) and those which remained lean (diet-resistant, DR).
Methods
Wistar-Rats were fed *ad libitum* with a high fat diet (HF, 40 energy% of fat) for 12 weeks. Weight changes and food intake as well as serum and cerebral spinal fluid (CSF) glucose, insulin, leptin and adiponectin were monitored during the diet phase. Using these parameters, a model for the identification of DIO rats was established. Next, gene expression profiles were obtained using Affymetrix GeneChips from the *M. gastrocnemius* of DIO and DR rats after one week of HF diet, i.e. before induction of obesity.
Results
No clear association between the animals' weight curves and any serum or CSF parameter was noticed, whereas the ratio between the absolute weight gain after one week and the rats' initial weight (weight gain index, WGI) was significantly correlated with the final weight and could therefore be used to predict obesity susceptibility. In skeletal muscle of DIO rats we detetected a striking transcriptional upregulation of genes from the MAP (mitogen acivated protein)-kinase pathway, intracellular calcium-sensing, pyruvate metabolism and of Rab-asscociated proteins participating in vesicle transport and GLUT4 translocation.
Conclusion
The prediction of susceptibility to diet-induced obesity is possible using simple auxologic parameters. Individual differences in skeletal muscle gene expression could contribute to the development of the DIO/DR-phenotype.

P495

A pharmacologic sympathectomy prevents the development of obesity and insulin resistance in the high fat fed rat

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Background
Obese patients show an increased activity of the sympathetic nervous system, but the causality of the relationship is not known. Here, we examine whether a pharmacologic sympathectomy influences diet-induced obesity and insulin resistance in the high fat fed rat model.
Methods
Wistar-Rats were fed *ad libitum* with a high fat diet (HF, 40 energy% of fat, based on lard) for 12 weeks. A pharmacologic sympathectomy was performed by a) weekly intraperitoneal applications of 6-hydroxydopamine (S1, $n=6$) or b) a single application of a saporin-bound dopamine-β-hydroxylase-antibody (S2, $n=6$) and verified by spleen immunohistochemistry. Insulin sensitivity was assessed by insulin tolerance tests and measurement of fasting glucose and insulin levels.
Results
Sympathectomy was successful in both experimental groups. Weight gain was reduced by 25% (S1, $P\leq0.05$) and 30% (S2, $P\leq0.05$), respectively, when compared to untreated controls. Fasting glucose did not differ significantly between the S1/S2 groups and the controls. Serum fasting insulin was reduced by 70% in S1 ($P\leq0.05$) and by 75% in S2 ($P\leq0.05$), respectively. Consistently, the in vivo insulin tolerance tests revealed an improved glucose disposal in the S1 and S2 animals when compared to the controls.
Conclusions
A pharmacologic sympathectomy can impede the development of obesity and insulin resistance in the high fat diet animal model. This effect could be used for the generation of new therapeutic strategies against the metabolic syndrome.

P496

Lipid-loaded hepatocytes release soluble factors that activate hepatic stellate cells: a new *in vitro* model to study fibrogenesis in NASH

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Background
Non-alcoholic steatohepatitis (NASH) can progress to hepatic fibrosis and end-stage liver disease. Hepatic stellate cells (HSC) are the central mediators of liver fibrosis. The molecular mechanisms linking hepatic steatosis to activation of HSC, thereby promoting inflammation and fibrosis, are mainly unknown. Here, we demonstrate a novel *in vitro* model to study the effect of hepatic lipid accumulation on HSC.
Methods and results
After exposure of human hepatocytes to the saturated fatty acid palmitate (PA), significant intracellular lipid accumulation was documented morphologically and by colorimetric assays. Subsequently, conditioned-media (CM) from PA-treated hepatocytes were used for stimulation of HSC, leading to enhanced proliferation and induction of the activation process of HSC. Furthermore, CM from PA-treated cells significantly induced a pro-inflammatory (MCP-1) and fibrogenic gene expression (Collagen I, TGF-beta, TIMP-1/-2, MMP-2) in HSC. Flow cytometric analysis revealed a significant apoptosis resistance of HSC after stimulation with CM from steatotic hepatocytes.
Summary and conclusion
Lipid accumulation in hepatocytes causes the secretion of soluble mediators that stimulate activation and proliferation of HSC as well as the expression of pro-inflammatory and fibrogenic genes in HSC. These findings demonstrate a potential mechanism how hepatic steatosis contributes to the progression of inflammation and fibrosis in NAFLD. Furthermore, this study provides an attractive *in vitro* model to identify innovative anti-fibrotic strategies for the therapy of NAFLD/NASH.

P497

Insulin resistance, atherogenic dyslipidemia and other atherogenic factors in simple obesity and obesity with Acanthosis nigricans (an) in childhood

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The aim of the study was to investigate insulin resistance, dyslipidemia and atherogenic factors influencing HDL functions in symple obesity (SO) and obesity with acanthosis nigricans (OAN). Althogether 37 children with obesity, 17 girls and 20 boys, 19 with SO and 18 with OAN, were included into the study. Their age was 14.2 ± 1.8 years and BMI was 35.9 ± 6.1 kg/m^2.
Fasting glucose (FG), insulin (INS) were measured and HOMA-IR was calculated. Total cholesterol (T-C), LDL-C, HDL-C, triglycerid (Tg), apoproteins (Ap-AI and AP-B100), lipoprotein a) /Lp(a)/were measured, and paraoxonase (PON-1), arylesterase (ARYL), lecitin cholesterol acyltransferase (LCAT), cholesterol-ester transfer protein (CEPT) activities, intracellular adhesion molecule-1 (ICAM-1) and vascular adhesion molecule (VCAM) concentrations were determined.
FG was normal in all obese patients, hyperinsulinemia was detected in 27, insulin resistance in 31 cases of 37 children. Increased T-C and LDL-C in 5–5, increased Tg in 6 and decreased HDL-C in 9 patients were found, abnormal Ap-AI in 13, Ap-B100 in 5, Lp(a) in 11 cases were detected. Frequencies of abnormal INS, Tg and HDL-C values were higher in OAN than in SO. PON1, ARYL, LCAT and CEPT activities as well as ICAM-1 and VICAM-1 did not differ. ICAM-1 levels were higher in patients with low HDL-C than in cases with normal HDL-C, and a significant negative correlation was found between PON-1 and ICAM-1.
These results demonstrate that insulin resistance and atherogenic dyslipidemia are more frequent in OAN than SO, and they also suggest that atherogenic dyslipidemia in childhood obesity has a complex influence to the parameters related to HDL function.

P498

Tribbles pseudokinases and adipose tissue biology

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Obesity, insulin resistance and high cholesterol levels are hallmarks of the metabolic syndrome. The molecular players involved in the onset of the metabolic syndrome are mostly unknown. In this context the three pseudokinases of the tribbles family have emerged as potential candidates. Among them, TRB3 has been described as a negative regulator of Akt, a key player in insulin signal transduction. However, the biological functions of TRB family members remain largely unknown. Therefore, the aim of this study was to learn more about the metabolic role of the three tribbles orthologs.
Analysis of tribbles mRNA expression in wt C57Bl/6J, db/db and ob/ob mice under fasting and refed conditions revealed that in both diabetic animal models the expression of TRB1 is significantly upregulated in adipose tissue. The expression of TRB3 is upregulated due to fasting conditions only in wt and db/db and the expression of TRB2 was not altered under any condition. Furthermore, in adipose tissue of C57Bl/6J mice treated with either dexamethasone for three weeks or with LPS for eight hours an upregulation of TRB1 and a downregulation of TRB3 could be observed compared to control animals, pointing to a role of TRB1 in the control of adipose tissue metabolism. To test for the cell autonomy of these effects, 3T3-L1 adipocytes were treated with different stimuli and analyzed for TRB expression patterns. Whereas, TRB1 expression was found to be upregulated due to insulin, dexamethasone, and LPS, TRB3 expression was downregulated.
The opposite regulation of TRB1 and TRB3 might be indicative of a role for these pseudokinases in the control of adipose tissue biology. Therefore, future experiments will address TRB-dependent signalling pathways in these cells and investigate their impact on the obesity phenotype.

P499

Differences in the POMC methylation pattern in normal weight and obese individuals and regulation of MC-4R promoter activity by DNA methylation

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The highly conserved Leptin-Melanocortin signaling pathway plays a central role in the regulation of body weight and energy expenditure. Mutations in POMC and MC-4R lead to dosage dependent monogenetic obesity. However, several evidences suggest an epigenetic impact on the regulation of body weight. Moreover it has been shown recently that variants of the FTO gene, which affects ss DNA methylation in the arcuate nucleus, correlate with increased body weight in children and adults. We therefore investigated the epigenetic state of the MC-4R and POMC gene in different, obese and normal individuals, in different tissues and in different species. We analysed the DNA methylation pattern of the POMC and MC-4R gene with bisulfite cloning technique in human peripheral blood cells, microdissected human ß-MSH expressing cells in the arcuate nucleus and in mice peripheral blood cells. Promoter activity has been analysed with Luciferase-Reporter-Gene-Assays. Comparing DNA from non-expressing peripheral blood cells and expressing arcuate neurons we observed a non-tissue specific POMC DNA methylation pattern, which was also conserved in mouse peripheral blood cells. We obtained a significant over-all hypermethylation in one CpG island of the POMC gene in peripheral blood cells in obese compared to normal weight individuals. In *in vitro* studies we could demonstrate that expression of the MC-4R gene was decreased after methylation of the promoter fragment. This study reveals a distinct evolutionary conserved DNA methylation pattern of the POMC and MC-4R gene, suggesting a functional role of methylation in melanocortin gene function. In aggreement with that the MC-4R promoter can be silenced by DNA methylation *in vitro*. In obese individuals we observed significant differences in the DNA methylation pattern of the POMC gene, which might lead to altered gene function and might contribute as an epigenetic alteration to the onset of obesity.

P500

Retinol-binding protein 4 (RBP-4) levels do not change after oral glucose tolerance test in obese and overweight individuals, but correlate with some indices of insulin resistance

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Background
Secretory products from adipocytes may contribute to increased insulin resistance (IR). Retinol-binding protein-4 (RBP-4) may increase IR in mice, with elevated levels in insulin resistant mice and humans with obesity and type 2 diabetes. Mechanisms regulating RBP-4 synthesis remain not fully understood. It is not clear whether short-term hyperglycaemia alters RBP-4 levels in humans. In order to investigate this, we measured serum RBP-4 concentrations during 75 gram oral glucose tolerance test (OGTT).
Subjects and methods
The study included 24 subjects (5 males), age 38.7 ± 15.1 years, BMI 34.4 ± 8.3 kg/m^2. Glucose, insulin and RBP-4 were measured at 0, 60 and 120 min of OGTT. IR was assessed by HOMA (calculated as fasting insulin (μU/ml)$\times$fasting glucose (mmol/l)/22.5) and by Insulin Resistance Index (IRI) that takes into account glucose and insulin levels during OGTT. IRI is calculated through the formula: $2/(1/(\mathrm{INSp} \times \mathrm{GLYp})) + 1$, where INSp and GLYp are the measured insulin and glycaemic areas.
Results
Glucose administration resulted in significant increases in insulin and glucose ($P < 0.0001$). There was, however, no change in RBP-4 concentrations (124.1 ± 32 μg/ml at 0 min, 123 ± 35 μg/ml at 60 min and 126.5 ± 37.5 μg/ml at 120 min of OGTT, P=ns). RBP-4 correlated moderately with fasting insulin ($r = 0.40$, $P = 0.025$), fasting glucose ($r = 0.41$, $P = 0.02$) and HOMA ($r = 0.43$, $P = 0.015$), but not with IRI ($r = 0.19$, $P = 0.31$). There was, however, only a moderate correlation between HOMA and IRI ($r^2 = 0.24$; $P = 0.006$) (Spearman rank correlation), while the best correlation was obtained between the product of glucose and insulin levels at 60 min of OGTT and IRI in a non-linear model ($r^2 = 0.88$; $P < 0.00001$).
Conclusions
RBP-4 levels do not change during oral glucose tolerance test, so it is unlikely that RBP-4 is involved in short-term regulation of glucose homeostasis. Moderate correlation between RBP-4 and HOMA suggests, however, that RBP-4 may be

one of many factors that influence insulin sensitivity in humans. Lack of correlation between RBP-4 and IRI may be a consequence of a limited correlation between static (HOMA) and dynamic indices of insulin resistance (IRI).

P501

Overweight adolescents, a group at risk for metabolic syndrome (Tehran Adolescent Obesity Study)

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Background
Metabolic syndrome not only is a serious problem for adults, but is also afflicting an increasing number of children and adolescents. This syndrome is a risk factor for type 2 diabetes mellitus and cardiovascular diseases. The aim of this study was to estimate the prevalence of metabolic syndrome in a sample of Iranian adolescents.
Methods
A total of 554 overweight adolescents (aged 11–17 years) participated in a community-based cross sectional survey. Anthropometric examinations including height, weight, body mass index, and blood pressure were assessed. A fasting blood sample was taken for measurement of glucose and lipid profile. Metabolic syndrome was determined by the definition released by the National Cholesterol Education Program Adult treatment Panel III, which was modified for age.
Results
The overall prevalence of metabolic syndrome was 26.6%. There was no gender difference in the distribution of metabolic syndrome. When stratified by body mass index, 22.5% of the overweight (BMI $\geq$ 95th percentile) adolescents met the criteria for MS, while 4.1% of the adolescents who were at risk for overweight (BMI between 85th and 95th percentile) had MS ($P<0.001$). Hypertriglyceridemia was the most common and low high density lipoprotein (HDL) was the least common constituent of metabolic syndrome.
Conclusion
This study suggests a high prevalence of metabolic syndrome among overweight Iranian adolescents. This poses a serious threat to the current and future health of Iranian youth.

P502

The obesity, physical activity status and dietary pattern in 10–12 years old girls of a mountainous region in north of Iran

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Background
It has been shown that one of major causes of obesity in young people could be explained by physical inactivity and fat intake.
Purpose
This study was carried out to investigate the prevalence of obesity, dietary pattern and physical activity status in Tonekabon girls, a mountainous city in north of Iran.
Methods
In a cross-sectional study 311 girls aged 10–12 years old in Tonekabon were studied. Weight, height, waist and hip circumferences of subjects were measured. Body mass index (BMI) and WHR (waist-to-hip ratio) were calculated. Food intake was assessed by using three 24-hour dietary recall and food frequency questionnaire. Physical activity level was measured using the physical activity questionnaire. Subjects were classified based on the intensity of effort as having light, moderate, heavy and very heavy levels of physical activity.
Results
The prevalence of overweight and obesity was 17 and 6%, respectively. 65% of subjects had normal weight and 12% were underweight. In 30% of the participants WHR was $\geq$0.85.The mean percentage values of energy intake derived from carbohydrate, protein and fat were 60, 11 and 29%, respectively. The physical activity level of subjects was 45, 43 and 12% that was light, moderate and heavy, respectively.
Conclusion
This study showed that overweight and obesity is common in this population. Thus prevention of overweight and obesity through a healthy diet and increasing the physical activity programs should be considered. In addition, the educational program to improve nutritional knowledge of this population is essential.

P503

Expression of the different components of the endocannabinoid system (ECs) in morbid obesity

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Visceral fat (VAT) may represent the more important site of endocannabinoid dysregulation in obesity. No data are available on the expression of the ECs in morbid obesity. Eleven morbidly obese women, who underwent bariatric surgery, were enrolled and paired samples of VAT and subcutaneous fat tissue (SAT) were obtained from each patient. Real-Time PCR for the gene expression levels of cannabinoid type 1 (CB1) receptor, *N*-acyl-phosphatidil-ethanolamine (NAPE), and fatty acid amide hydrolase (FAAH) which respectively sinthesize and degradate anandamide (AEA) and, diacylglycerol lipase (DAGL-β) and monoacylglycerol lipase (MAGL), which respectively synthesize and degrade 2-arachidonoyl-glycerol (2-AG) was performed. After extraction and after exclusion of genomic DNA contamination, 1 μg RNA was reverse transcribed using oligodT primers. Real-time cDNA quantification was performed by a thermocycler. iCycler iQ® (BioRad). CB1, FAAH, NAPE-PLD, DAGL-β and MAGL primers for SYBR® Green analysis were designed by 'Beacon Designer®' and synthesized by Invitrogen. Assays were performed in duplicate and a standard curve from consecutive 10-fold dilutions of a cDNA pool representative of all samples, was included for each determination. Relative expression analysis was corrected for PCR efficiency and normalized respect to reference gene β-actin. BMI was 46.3 ± 1.38 kg/m^2. Total cholesterol was 186 ± 12.8 mg/dL, HDL-cholesterol 53.3 ± 3.74 mg/dL and triglycerides 118 ± 16.3 mg/dL. CB1 mRNA was significantly higher in SAT than in VAT. VAT had a significantly higher expression of NAPE than SAT. There was no difference in FAAH mRNA between SAT and VAT. SAT displays a higher expression of MAGL than VAT, not reaching the statistical significance. DAGL-β was more significantly expressed in SAT than in VAT. These data, obtained in morbid obesity, suggest that the ECs plays a crucial role not only in VAT but also in SAT, underlying the differences in the ECs dysregulation when morbid obesity without metabolic alterations is compared to abdominal obesity with metabolic alterations.

P504

Plasma adrenomedullin positively correlates to body mass index, and significantly decreases after bariatric surgery

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Adrenomedullin is a vasoactive peptide originally discovered in extracts of human pheochromocytoma. It is highly expressed in plasma and adipose tissue of obese subjects and considered as a member of the adipokine family. Adrenomedullin inhibits adipogenesis under the transcriptional control of insulin. We determined plasma adrenomedullin concentrations in a cohort of 360 healthy subjects and found a very significant positive correlation to body mass index (BMI). In parallel, 28 morbidly obese patients were selected from the cohort scheduled to undergo laparoscopic gastric bypass surgery, and studied at two time points: just before and one year after surgery. As expected, bariatric surgery induced significant decreases in body weight, BMI, insulin resistance, plasma leptin, total cholesterol, LDL-cholesterol and CRP. Plasma adrenomedullin levels decreased one year after bypass surgery in all the subjects studied: preoperative adrenomedullin was 0.757 ± 0.033 and postoperative values were 0.624 ± 0.025 ($P<0.001$). In obese subjects, basal plasma adrenomedullin significantly correlated with leptin. Moreover, there was a positive association of surgery induced changes in plasma adrenomedullin and leptin. In summary, we present here a positive significant correlation of adrenomedullin with BMI in healthy patients. Weight loss after bariatric surgery is associated with a significant decrease in plasma adrenomedullin.

P505

Plasma osteopontin correlates with insulin resistance in obese subjects, but increases following bariatric surgery parallel to other markers of bone turnover

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Osteopontin (OPN) is a multifunctional glycoprotein implicated, among others, in bone metabolism, cardiovascular disease, diabetes and obesity. It promotes inflammation and insulin resistance. Osteopontin is elevated in the plasma and adipose tissue of obese subjects and decreases upon diet induced weight loss. Here we investigated the effect of bariatric surgery on plasma OPN concentrations in morbidly obese patients. 40 obese patients aged 43.1 ± 1.8 years were recruited from the cohort scheduled to undergo bariatric surgery. Roux-en-Y gastric bypass (RYGB) was performed in 30 subjects (27 females, 3 males), and laparoscopically adjustable gastric banding (LAGB) in 10 subjects (8 females, 2 males). All patients were studied before and one year (10.3 to 14.8 months) after the surgical intervention. Prompted by the associations of OPN with both bone metabolism and metabolic diseases, we explored the changes in metabolic, inflammatory and bone turnover parameters. Both bariatric procedures significantly reduced body weight, body mass index (BMI), insulin, leptin and CRP one year after surgery. Plasma OPN increased from 31.38 ± 3.8 to 52.81 ± 3.7 ng/ml after RYGB ($P < 0.001$) and from 29.76 ± 6.9 to 46.4 ± 10.6 ng/ml after LAGB ($P = 0.042$). Preoperative OPN correlated with age, insulin, HOMA insulin resistance index and postoperative OPN. Postoperative OPN correlated with C-telopeptide and OC. In summary, plasma OPN levels significantly rise and correlate with biomarkers of bone turnover one year after RYGB and LAGB. Unlike other pro-inflammatory cytokines, OPN does not normalize, but further increases after bariatric surgery.

P506

Cholinergic regulation of meal induced ghrelin and PYY release is impaired in obesity

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Ghrelin and PYY are gastrointestinal peptides involved in appetite regulation. The cholinergic part of the vagal nerve participate in the regulation of glucose and insulin levels. We aimed to examine the effects of the cholinergic antagonist atropine on ghrelin, PYY, glucose and insulin under basal conditions and after meal ingestion in lean and obese subjects. Eight lean and eight obese subjects received in a randomized, double-blind, placebo controlled crossover study design (1) placebo, (2) placebo + breakfast, (3) atropine and (4) atropine + breakfast. Plasma ghrelin, PYY, insulin and glucose were measured. Hunger and satiety feelings were rated on 10 cm visual analog scales. In lean individuals atropine led to a decrease in plasma ghrelin and to a significant decrease in both basal and meal induced PYY concentrations. In obese subjects atropine did not significantly change ghrelin or PYY concentrations whereas it induced a comparable increase in heart rate and in meal induced glucose concentrations in the two study groups. Only lean, but not obese subjects experienced sustained feelings of satiety after breakfast. We suggest that the impaired cholinergic regulation of the postprandial drop in ghrelin concentrations and rise in PYY concentrations might be part of the deregulated food intake in obese subjects.

P507

Serum adiponectin levels in children with nonspecific infections: relations with fever and acute phase reactants

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It has been shown that adiponectin (AN) has antidiabetic, antiatherosclerotic and antiinflammatory activities. We analyzed serum AN levels in children with nonspecific infections. Forty-two prepubertal children (18 boys, 24 girls, age range: 14–163 months) admitted with fever ≥ 38 °C and nonspecific infection (upper respiratory tract infection, otitis, urine tract infections) symptoms were included. Their ideal body weights (IBW) for height were within 100–110%. Venous blood samples were taken from them both during the period of the fever at admission and three days later after the fever dropped (control) for analyzing serum AN levels and complete blood count (CBC), erythrocyte sedimentation rate and CRP, Hs-CRP, fibrinogen, ferritin, glucose, insulin, total cholesterol, triglyceride, LDL-C, HDL-C levels. Serum AN levels at the admission (8.8 ± 1.7 μg/ml) were lower than at the control visit (12.04 ± 2.38 μg/ml)($P < 0.0001$). Serum AN levels were not correlated with the degree of fever, BW, IBW, body-mass index, homeostasis model assessment (HOMA-IR), and the all other parameters analyzed in the study ($P > 0.05$) in both analysis except glucose/insulin ratio at the admission ($r = 0.33$, $P < 0.05$). Serum glucose levels, glucose/insulin and HDL-C levels were higher ($P < 0.01$, $P < 0.05$ and $P < 0.001$, respectively) and triglyceride and LDL-C levels were lower ($P < 0.001$, $P < 0.01$, respectively) at the admission than at the control. Lower serum AN levels were found in children with fever during the acute phase of nonspecific infections. It is suggested that AN may have anti-inflammatory and acute phase reactant roles in infections.

P508

NCEP-ATP-III defined metabolic syndrome, type 2 diabetes mellitus and prevalence of hypogonadism in male patients with sexual dysfunction

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Objectives
Type 2 diabetes mellitus (T2DM) and metabolic syndrome (MetS) are characterized by insulin resistance and often associated with male hypogonadism. To discriminate the specific contribution of T2DM and MetS to male hypogonadism.
Design and methods
A consecutive series of 1134 (mean age 52.1 ± 13 years) male patients with sexual dysfunction was studied. Several hormonal and biochemical parameters were studied along with ANDROTEST, a 12-item validated structured interview, specifically designed for the screening of hypogonadism (total testosterone TT, < 10.4 nmol/l or free-testosterone FT, < 37 pmol/l) in a male population with sexual dysfunction.
Results
Irrespective of the criteria used to define hypogonadism, MetS was associated with a significantly higher prevalence of the condition, both in subjects with and without T2DM (41 and 29% vs 13.2% and 77.1 and 58% vs 40.6%; respectively for TT and FT in patients with MetS and with or without T2DM, when compared with subjects without MetS and T2DM; both $P < 0.0001$). Conversely, T2DM was associated with a higher prevalence of hypogonadism in subjects with MetS but not in those without MetS. Patients with MetS, with or without T2DM, also showed a higher ANDROTEST score when compared with patients without MetS. Logistic multivariate regression analysis, incorporating the five components of MetS, identified a significant association of elevated waist circumference and hypertriglyceridaemia with hypogonadism both in patients, with or without T2DM.
Conclusions
Our study demonstrated that MetS and in particular visceral adiposity (as assessed by increased waistline and hypertriglyceridaemia) is specifically associated with hypogonadism in subjects consulting for sexual dysfunction.

P509

Interleukin-1ß is a positive regulator of TIARP/STAMP2 in 3T3-L1 adipocytes

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Tumor necrosis factor alpha-induced adipose-related protein (TIARP)/six-trans-membrane protein of prostate 2 (STAMP2) has been characterized as an adipocyte-expressed protein which might play a crucial role in metabolic homeostasis and inflammatory pathways. In the current study, the impact of insulin resistance-inducing and proinflammatory interleukin (IL)-1ß on TIARP/STAMP2 gene expression was determined by quantitative real-time reverse transcription-polymerase chain reaction in

3T3-L1 adipocytes. Interestingly, TIARP/STAMP2 mRNA synthesis was significantly stimulated by IL-1ß in a dose-dependent fashion with 2.6-fold induction seen at IL-1ß concentrations as low as 0.02 ng/ml and maximal 9.2-fold upregulation found at 20 ng/ml effector. Furthermore, induction of TIARP/STAMP2 mRNA by IL-1ß was time-dependent with maximal 18.6-fold upregulation detectable after 8 h of IL-1ß treatment. Signaling studies suggested that janus kinase 2, nuclear factor κ B, and p44/42 mitogen-activated protein kinase are involved in IL-1ß-induced TIARP/STAMP2 mRNA expression. Taken together, these results show that TIARP/STAMP2 is highly upregulated in fat cells by IL-1ß and might participate in proinflammatory and insulin resistance-inducing effects of this cytokine.

P510

Daily and nightly urinary free cortisol ratio as a marker of the hypothalamic-pituitary-adrenal (HPA) axis activity in abdominal obesity

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Abdominal obesity (AO) might have a hyperactivation of the HPA axis but previous studies are limited by the small and heterogeneous number of patients investigated. The aim of this study was to evaluate urinary free cortisol (UFC) output during daily and nightly hours in a large cohort of AO women versus normal weight controls (CT). 107 AO women and 37 CT women were enrolled in this study; all subjects underwent a complete physical examination, an OGTT and biochemical determinations. Moreover, each subject collected daily (from 0800 am to 0800 pm, dUFC) and nightly (from 0800 pm to 0800 am of the day after, nUFC) urine for UFC determinations.
Total cholesterol and triglycerides levels were significantly higher in the AO, whilst HDL were significantly lower than in CT. AO had significantly higher HOMA index than CT. There were no differences neither in dUFC nor in the nUFC between the groups but on the contrary, AO had significantly lower dUFC/nUFC ratio than CT. There was a negative and significant correlation between dUFC/nUFC and waist and BMI in all subjects. In the AO group, the correlation between dUFC/nUFC and anthropometric variables was still present, moreover, the ratio was also positively correlated to HOMA index.
In order to assess the linkage between HPA axis activity and metabolic syndrome, a multiple regression was performed in AO. dUFC/nUFC was still negatively and significantly correlated to BMI, while the correlation with waist circumference was lost. Interestingly, dUFC/nUFC was still positively and significantly correlated to HOMA index and systolic blood pressure. On the contrary, a negative and significant correlation was found between dUFC/nUFC and both HDL and diastolic blood pressure.
In conclusion, obesity by itself is characterized by high nightly UFC excretion. The HPA axis dysregulation is strictly associated to the abnormalities of the metabolic syndrome.

P511

Impact of dietary management and diet duration on body weight and metabolic and endocrine changes in rats fed a high fat/low carbohydrate diet

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High-fat/low carbohydrate (HF–LC) diets in humans, such as the Atkin's diet, are claimed to work in an ad libitum setting. In a previous study, we had demonstrated reduced bodyweight (BW) gain in rats when pair-fed isocaloric amounts of a HF–LC compared to normal chow (CH). However, rats on HF–LC also showed increased body fat and low serum IGF-I. We now compared BW development, food intake and resting energy expenditure (EE) in an ad libitum setting in male Wistar rats fed a HF-LC or CH matched in protein content ($n=8$ per group). After 20 days (d) the HF–LC group had gained significantly more BW (HF-LC: 41.1±7.1 g versus CH: 27.1±13.8 g; $P=0.02$). BW-gain in the HF–LC group was significantly higher between d10 and d20 than between d1 and d10 (d1–10: 15.5±6.1 g; d10–20: 25.6±6.7 g; $P<0.01$) despite comparable caloric intake. CH fed controls gained similar weight in both intervals (d1–10: 12.4±9.1 g; d10–20: 14.7±10.4 g; $P=0.65$). No differences in EE were observed between the groups. EE did not change with duration of the diet, but the respiratory exchange ratio continuously decreased on HF–LC group. Daily energy excess was greater in the HF–LC group (CH: 18.95±6.97 kcal; HF–LC; 39.71±7.6 kcal; $P<0.01$). HF–LC fed rats have severely reduced daily faeces amounts (CH: 9.53±1.5 g versus HF–LC: 2.92±0.3 g; $P<0.001$), remaining energy in the faeces is under investigation. Preliminary results indicate that IGF-I levels are also reduced with ad libitum access to HF–LC. In conclusion, the weight loss observed on HF–LC diet with pair feeding was not seen in an ad libitum setting, but changes in metabolic parameters and IGF-I seem to be similar. Also in humans reduced palatability and availability of food items on HF–LC diets might lead to a reduced caloric intake and thus explain the observed weight loss.

P512

No feedback inhibition of obestatin during acylated ghrelin infusion in humans

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Background
Obestatin is a 27 aa peptide derived from the ghrelin gene. Obestatin was first described to exert anorexigenic effects by decreasing gastric motility in rodent models, however these results have been debated and there is presently only small information regarding its activity and its regulation in humans. Furthermore, its interactions with acylated ghrelin (AG), derived from the same gene, have not been evaluated. Therefore, in these preliminary data, we sought to evaluate obestatin levels in normal subjects submitted to a 2 h infusion with 1 μg/kg per h AG or isotonic saline.
Methods
Three normal subjects were included in the study. An isotonic saline infusion was maintained for 1 h and then, either AG (1 μg/kg per h) or isotonic saline was continuously administrated iv for 2 h. Blood samples were collected at times −60, −30, 0, 30, 60, 90 and 120 min. For each treatment, infusion, peak, nadir and AUC obestatin values were evaluated. Glycemia and insulin levels were evaluated for each timepoints.
Results
Baseline circulating obestatin concentrations were respectively 246±102 ng/ml and 242±111 ng/ml for the 2 treatment groups (isotonic saline and AG infusions). Furthermore, obestatin levels were not significantly modulated by the administration of either isotonic saline (mean value=267±56 ng/ml) or AG (mean value=270±81 ng/ml). These results suggest that both observed insulin and obestatin circulating concentrations could be correlated ($r=-0.60$; $P=0.59$).
Conclusion
This preliminary study suggests that obestatin levels are not regulated by a feedback mechanism during an AG infusion in normal subjects. Meanwhile, the possibility of an association between obestatin and insulin concentrations could be investigated in further studies.

P513

Prevalence and characterization of metabolic syndrome (MS) in different classes of obesity and related endocrine factors

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The aim of study was to evaluate in a group of obese women the prevalence of the metabolic syndrome, defined accordingly to the International Diabetes Federation, of single factors of the syndrome and the correlations with endocrine factors. We evaluated 351 women, 262 overweight-obese and 89 normal weight controls, which underwent physical, biochemical and hormonal evaluation. The prevalence of MS in obese and overweight subjects was 62.3%; the prevalence increased significantly by increasing body mass index, ranging from 20.6% for overweight to 71.6% for class III obesity. The analysis of prevalence of single factors of MS showed that the prevalence of low HDL levels and high fasting glycaemia had an inverse trend, by increasing values from overweight to class II obesity but decreasing levels in class III obesity. In the normal-weight controls there is 12.5% prevalence of increased waist circumference and 14.8% prevalence of hypertension. The analysis of the prevalence of single factors showed that in overweight subjects the commonest association was between waist circumference, high fasting glycaemia and hypertension. In class I and II obesity, the most frequent association was between metabolic parameters whereas in class III obesity, hypertension was the main element. Urinary free cortisol and testosterone levels were significantly higher in subjects with MS.
In conclusion, the lower prevalence of metabolic alterations in massive obesity might be related to genetic factors or to a protective role of the large amount of subcutaneous fat of these individuals. The presence of some elements of the MS also in normal weight controls indicate that even this group had a risk of development of MS. Hormonal differences detected in women with MS might be due to the already described different hypothalamic-pituitary-adrenal axis activity and/or to the effect of insulin resistance.

P514

Relationship among stress, weight gain and the activity of the hypothalamic-pituitary-adrenal (HPA) axis

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The aim of this study was: 1) to determine whether there is a correlation between a stressful event, such as the mourning, the hyperactivity of HPA axis, and the fast weight gain 2) to assess whether there is a specific parameter that can predict the susceptibility of individuals to develop metabolic syndrome following a stressful event. We investigated 80 obese women, aged between 23 and 67 years; each subject underwent a clinical evaluation, an OGTT and biochemical, metabolic, hormonal and inflammatory parameter determinations. To express the amount of time in which weight gain has happened, an arbitrary index called INCR (ratio between weight gain and interval time) was used. Subject were divided into two groups based on the history of their weight increase: the first group, used as controls, included 61 women become obese dynamically, after pregnancy; the second group, called Mourning, however, included 19 women become obese after mourning. The parameter INCR was significanthy different between two groups. There were no significant differences between the groups neither in metabolic profile nor in insulin resistance indices. The Mourning group had significantly higher levels of 24hrs-FCU than the control group. The values of androstenedione were significantly higher in the Mourning group compared with controls. All other hormonal parameters were not significantly different between two groups. This study confirm that women become obese following an acute stress showed an hyperactivation of the HPA axis as documented by higher 24 h-UFC and androstenedione levels, which support the hypothesis that acute stress may be involved in fast weight increase. The lack of difference in metabolic parameters might be due or to the small time of observation or to the small number of subjects. However, 24 h-FCU and androstenedione levels might be considered as predictive parameters of future metabolic alterations.

P515

Glucose-dependent insulinotropic polypeptide (GIP) receptor knock-out prevents ovariectomy-induced obesity in mice

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Objectives
Premature estrogen deficiency and menopause are associated with increases in body weight and fat mass. Ovariectomized (OVX) mice also show reduced locomotor activity. Because glucose-dependent-insulinotropic-polypeptide (GIP) is known to play an important role both in fat metabolism and locomotor activity, we hypothesized that effects of estrogen on the regulation of body weight, fat mass and spontaneous physical activity could be mediated in part by GIP signaling.
Methods
C57BL/6 mice and GIP-receptor knock-out mice (Gipr−/−) were exposed to OVX or sham-operation ($n=10$ per group). Effects on body composition, energy expenditure, locomotor activity, markers of insulin resistance, and expression of hypothalamic anorexigenic and orexigenic factors were investigated over 26-weeks in all four groups of mice.
Results
OVX wild-type mice developed obesity, increased fat mass, and elevated markers of insulin resistance as expected. This was completely prevented in OVX Gipr−/− animals, even though their spontaneous locomotor activity levels and energy expenditure did not significantly differ from those of OVX wild-type mice. Cumulative food intake in OVX Gipr−/− animals was significantly reduced and associated with significantly lower hypothalamic mRNA expression of the orexigenic neuropeptide Y (NPY). Hypothalamic expression of cocaine and amphetamine related transcript (CART) or thyroid stimulating hormone releasing hormone (TRH) were not significantly different in OVX Gipr−/− vs OVX wild-type control mice.
Conclusions
GIP receptors appear to interact with estrogens in the hypothalamic regulation of food intake. Pharmacological inhibition of GIP receptors may carry promising potential for the prevention of obesity in estrogen deficient states.

P516

Chronic central infusion of a ghrelin receptor (GHS-R1A) antagonist to rats: impact on ICV ghrelin-induced food intake and altered body composition

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Central treatment with ghrelin increases body weight and fat accumulation. Here we explore the pharmacological effect of a combined central (ICV) infusion of ghrelin (0.3 nmol/day) and a recently developed ghrelin receptor (GHS-R1A) antagonist (JMV2959, 100 nmol/day) on food intake and body composition. The effect of ghrelin infusion for 14 days to increase body weight ($20.7\pm0.9\%$ vs $34.3\pm3.1\%$, saline vs ghrelin, $P<0.001$) was blocked by co-infusion with JMV2959 ($34.3\pm3.1\%$ vs $24.8\pm2.5\%$, ghrelin vs JMV2959+ghrelin, $P<0.05$). The higher food intake (rat chow; 333.4 ± 6.8 g vs 407.4 ± 16.4 g, saline vs ghrelin, $P<0.005$) and the increase of food efficiency ($17.9\pm0.5\%$ vs $24\pm1.4\%$, saline vs ghrelin, $P<0.05$) in ghrelin treated rats was not suppressed by the antagonist (407.4 ± 16.4 g vs 368.3 ± 13.4 g and $24\pm1.4\%$ vs $19.8\pm1.6\%$. Body composition analysis (DEXA) showed that ghrelin treatment increased delta lean mass ($9.4\pm1.4\%$ vs $12.9\pm1.2\%$, saline vs ghrelin) and delta fat mass ($0.27\pm0.5\%$ vs $2.6\pm0.6\%$, saline vs ghrelin, $P<0.001$) but only the latter was blocked by JMV2959 ($2.6\pm0.6\%$ vs $-2.1\pm0.3\%$, ghrelin vs ghrelin+JMV, $P<0.001$). Ghrelin induced an increase in the weight of all dissected fat pads (retroperinotenal, inguinal, reproductive and mesenteric as well as the intracapsular brown adipose (iBAT) tissue) but only the effects on retroperitoneal, inguinal and iBAT depots were suppressed by the antagonist.
Thus, the ghrelin antagonist JMV2959 suppresses the weight gain and fat accumulation induced by central ghrelin administration. These antagonist effects are not mediated by changes in food intake and food efficiency and future studies will elucidate whether this is due to an influence on metabolic rate. *Supported by EC 6th LSHM-CT-2003-503041.*

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Increased unsaturated fatty acids biosynthesis when pyruvate carboxylase is suppressed in adipocytes

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To maintain a proper lipid composition of cellular membranes is fundamental for cell viability and intracellular signalling. Therefore we hypothesised the existence of membrane quality control homeostatic mechanisms. Previously, we have shown that Pyruvate Carboxylase (PC), an enzyme involved in *de novo* lipogenesis and fuel partitioning, is a target of PPARγ and that PC level is decreased in PPARγ KO and obese insulin resistant mouse models. Here, we used RNAi knock-down of PC (PC-KD) in 3T3-L1 preadipocytes to elucidate the metabolic consequences associated with the decreased levels of PC *in vitro*. Our results show that reduced mRNA expression and protein levels of PC (by 90 – 95%) does not prevent fat accumulation in 3T3-L1 adipocytes. However, using ^{13}C-Nuclear Magnetic Resonance spectroscopy and Gas Chromatography-Mass Spectrometry we found that suppressed levels and activity of PC changes the composition of fatty acid acids being synthesised resulting in increased amount of unsaturated fatty acids (fatty acids with structure 16:1, 18:1, 20:1), and decreased levels of saturated fatty acids (fatty acids with structure 10:0, 14:0, 15:0, 16:0, 22:0, 24:0). This was associated with specific gene expression (qRT PCR) changes of elongase enzymes (Elovl). Moreover, we show that the same pattern of reduction in PC expression and preferential accumulation of unsaturated fatty acids is also observed in adipose tissue of morbidly obese individuals compared to lean individuals (local ethical committee approval had been obtained). Therefore our results support the notion that that under metabolic dysregulation such as seen in obesity and insulin resistance, when there is decreased substrate flux for *de novo* lipid biosynthesis, adaptive mechanisms do occur to maintain the biosynthesis of unsaturated fatty acids. We speculate that this adaptation may ensure that enough unsaturated fatty acids are available to maintain membrane lipid composition.

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Glomerular filtration rates as a cardıometabolıc risk markers in obese women

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Introduction
This study carried out relationship between glomerulary filtration rates (GFR) and various metabolic parameters in obese women.
Materıals and methods
Selected subjects recruited retrospectively from outpatient clinic for this study were 5115 overweight or obese women with mean GFR which was 93 ml/dk/1.73 m^2 calculated by MDRD (modification of diet in renal disease). According to mean GFR, they divided group I (low GFR) having $\leq$92 ml/dk/1.73 m^2 and group II (normal GFR) having $>$93 ml/dk/1.73 m^2. Thereafter, we determined and compared body compositions (body mass index, abdominal fat mass), resting blood pressures, plasma lipoprotein levels, glucose homeostasis and other related biochemical parameters.
Results
Calculated GFR were 74.2$\pm$10.8 ml/dk/1.73 m^2 in low GFR group (n=2555, 49.9%) and 110.8$\pm$34.1 ml/dk/1.73 m^2 in normal GFR group (n=2560, 50.1%). Mean age, BMI, body fat mass, systolic and diastolic blood pressures, fasting glucose, fasting insulin, HOMA values, total cholesterol, LDL-cholesterol, triglycerides, uric acid and high sensitive C-reactive protein levels, counts of leukocytes, liver transaminases except aspartate transaminases activity were significantly higher in low GFR group than normal GFR group ($P<0.05$). But mean waist circumferences, abdomal fat mass and ferritin levels were not different between groups ($P>0.05$; Table 1).

Table 1

	Low GFR group (n=2560)	Normal GFR group (n=2555)	P values
Age (year)	43.47$\pm$11.93	37.31$\pm$11.63	<0.001
BMI (kg/m^2)	36.42$\pm$6.93	35.74$\pm$6.90	<0.001
Waist circumferences (cm)	100.68$\pm$13.55	100.32$\pm$19.50	NS
HOMA	3.81$\pm$4.95	3.32$\pm$4.17	0.008
Total cholesterol (mg/dl)	213.79$\pm$45.49	197.55$\pm$39.70	<0.001
Triglycerides (mg/dl)	162.66$\pm$126.98	134.46$\pm$113.34	<0.001

Discussion
In obese women, reduction in GFR was associated with metabolic, cardiovascular and renal end points. It should be careful during a slimming program with included GFR in obese or overweight women.

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In the metabolic syndrome adrenergic overdrive is independent on the obese state

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Objective
The metabolic syndrome (MS) is characterized by a marked sympathetic activation. Whether this adrenergic overdrive is mainly dependent on the obese state or it can be detected also in absence of obesity is unknown, however.
Design and Methods
In 25 male patients (age 41.4$\pm$yrs., mean$\pm$S.E.M.) with MS (ATP III criteria) and in 12 age-matched healthy male controls, we measured body mass index (BMI), waist circumference (WC), beat-to-beat arterial blood pressure (Finapres), heart rate (EKG), HOMA index and efferent post-ganglionic muscle sympathetic nerve traffic (microneurography, MSNA). Measurements were performed at rest and during arterial baroreceptor stimulation and deactivation via vasoactive drug infusion.
Results
Patients with MS were classified as obese (MSO, n=16, BMI 33.7$\pm$0.7 kg/m^2 and WC 109.2$\pm$1.3 cm) and lean (MSL, n=9, BMI 26.2$\pm$0.7 kg/m^2 and WC 96.3$\pm$0.7 cm). HOMA index was significantly greater in MSO than in MSL (5.1$\pm$0.3 vs. 3.9$\pm$0.3, $P<0.05$), while all the other variables of the MS were similarly altered in the 2 groups. Both MSO and MSL displayed MSNA values greater than C (62.4$\pm$1.5 and 52.8$\pm$1.7 vs 39.7$\pm$1.2 bs/100 hb, $P<0.05$). Compared to C, both the bradicardic and the tachycardic responses to vasoactive drugs were impaired in MSL ($-$24.4$\pm$4 and $-$29.5$\pm$6%, $P<0.05$), a further impairment being detected in MSO ($-$40.6$\pm$7 and $-$46.2$\pm$8%, $P<0.05$). This was the case also for sympathoinhibitory and sympathoexcitatory responses to baroreceptor manipulation (MSL $-$26.6$\pm$5 and $-$39.4$\pm$7%, MSO $-$39.8$\pm$8 and $-$51.1$\pm$6%, $P<0.05$ for all).
Conclusions
These data provide evidence that the sympathetic activation and the baroreflex impairment characterizing the MS are independent on the presence of obesity. They also show that the obese state exerts potentiating effects on the sympathetic alterations seen in the MS, presumably because of the greater autonomic (baroreflex impairment) and metabolic (insulin resistance) alterations seen when the obese state and the MS are combined together.

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Changes of the testosterone levels in young males with obesity and metabolic syndrome

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Aim
The present study aimed to compare the androgen levels in men with obesity, metabolic syndrome /MS/ and healthy normal – weight controls.
Materials and methods
Eighty-six males (mean age 29.65$\pm$8.57 /18–50/) were investigated. 31 of them were healthy (mean BMI – 23.66$\pm$1.97); 22 were with simple obesity (mean BMI – 32.79$\pm$7.16) and 33 (mean BMI – 35.78$\pm$8.71) were with overt metabolic syndrome /MS/ according to the IDF definition. The levels of the sex-hormone binding globulin /SHBG/, total /TT/ and free testosterone /FT/ were determined.
Results
The mean levels of the TT were lowest in the group with metabolic syndrome (16.34$\pm$7.36 nmol/l) and highest among healthy non-obese men (30.08$\pm$10.96 nmol/l). The mean androgen levels of the obese males (21.27$\pm$6.89 nmol/l) were significantly higher than in the MS group but significantly lower than in the healthy controls. The concentrations of the FT in the groups with obesity (0.50$\pm$0.18 nmol/l) and MS (0.46$\pm$0.20 nmol/l) were not significantly different. However, they both were lower in comparison to the levels in healthy men (0.69$\pm$0.27 nmol/l). The SHBG concentrations were lowest in the male with MS (20.74$\pm$12.94 nmol/l vs. 31.31$\pm$17.40 nmol/l in obese male and 36.84$\pm$16.25 nmol/l in controls).
Conclusion
In young males with obesity and MS the serum levels of androgens decrease. Probably, the reduction of the SHBG in patients with MS limits further decrease of the free testosterone, but it is also related to negative metabolic consequences. Further investigations are needed to clarify the possible mechanisms.

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Expression of adrenergic receptors and atrial natriuretic peptide in human adipose tissue of severe obese women and Rosiglitazone action during differentiation of human pre-adipocytes

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Background
The balance between Adrenergic Receptor (AR) subtypes and Atrial Natriuretic Peptide (ANP) is crucial for lipolysis. It was previously demonstrated that ANP is strongly lipolytic and we recently demonstrated that ANP is expressed and

secreted by human pre-adipocytes. In previous studies, β-receptor density and lipolysis were higher in human visceral adipose tissue (HVAT) as compared with human subcutaneous adipose tissue (HSAT).
Aims and Methods
The expression levels of ANP, AR subtypes and adipocyte differentiation markers (AdM) were analyzed by real-time RT-PCR in human subcutaneous (HSAT) and visceral (HVAT) fat biopsies from obese women and in a human pre-adipocytes cell line (ChubS7) during rosiglitazone-mediated differentiation.
Results
The expression of α2A-AR and β_2-AR were higher in HSAT than in HVAT; α1A-AR, β1-AR and ANP expression were comparable, and α1B-AR, α1D-AR and β3-AR were not measurable in HVAT and HSAT. In both rosiglitazone- and non-stimulated Chub-S7 all AR subtypes, except for β3-AR, as well as ANP were expressed from day 3 to 6 of differentiation. At day 17, in rosiglitazone-stimulated Chub-S7, ANP was switched off, AdM and β3- AR were switched on, β2-AR increased, β1-AR was constantly low and α2A-AR significantly decreased as compared with basal.
Conclusions
Data on HVAT and HSAT suggest that both the pro-lipolytic β2-AR and the anti-lipolytic α2A-AR are involved in the balance between HVAT and HSAT accumulation. Data on ChubS7 demonstrate that β3-AR and AdM are simultaneously switched on during adipocyte differentiation. We suggest that at the end of the rosiglitazone-mediated differentiation, adipocytes require β3-AR-activation whereas the α2A-AR anti-lipolytic and ANP-mediated effects are decreased.

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Glucose-dependent insulinotropic polypeptide downregulates expression and enzyme activity of 11β-Hydroxysteroid Dehydrogenase type 1

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Introduction
11β-hydroxysteroid dehydrogenase type 1 (11 β-HSD1) converts cortisone into its active metabolite, cortisol. Mechanisms regulating the promoter-activity of 11β-HSD1 are of considerable importance to understand the basics of intracellular cortisol, lipid and glucose metabolism. The incretine glucose-dependent insulinotropic polypeptide (GIP) has been suggested to affect insulin sensitivity. However, the mechanisms of this effect are unclear yet.
Objectives and Settings
We aimed to analyse the effect of GIP on 11β-HSD1 enzyme activity and mRNA level in adipose tissue. Therefore fat biopsies of 10 healthy overweight men (BMI: 28–40 kg/m^2; age: 30–65 years) with a normal glucose tolerance were taken before and after a GIP or saline infusion over 4 h. Enzyme activity of 11β-HSD1 was measured and qRT-PCR was performed to determine the gene expression level of 11β-HSD1. Effects of GIP on 11β-HSD1 promoter activity were analysed using 11β-HSD1-Luciferase promoter constructs in 3T3-L1 cells.
Results
GIP reduced mRNA-expression and activity by approximately 30% ($P<0.05$). Comparably, the promoter activity of 11β-HSD1 as measured by Luciferase activity and the mRNA-expression of 11β-HSD1 was substantially reduced by GIP in differentiated 3T3-L1 cells.
Conclusion
We demonstrated that GIP downregulates the expression and activity of 11β-HSD1 in adipose tissue *in vivo* and *in vitro*. Thus, GIP might affect fat metabolism via inhibition of 11β-HSD1.

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Obesity in GDM: a registry of GDM in Portugal

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Introduction
A retrospective study of the year 2003, of 1314 women with GDM, from 24 public health Centres in Portugal, was performed.
Patients and methods
Women were divided into two groups according to their pre-pregnancy BMI: group Go – BMI ≥ 30 kg/m^2 and group Gno BMI <30 kg/m^2. The mean age of these women was 32.9 ± 5 years (18–45), the A1c was $<6\%$ in both groups. The influence of the BMI in different variables was analysed: family history of DM, weight gain during pregnancy according to the recommendation of the Institute of Medicine – Washington 1990, blood pressure, need of insulin therapy, gestation age at the beginning of insulin therapy, time of delivery, type of delivery, new-born weight and the re-evaluation post-partum.
Results
The mean BMI was 26.7 ± 5.1 (16–49.7), 76.3% had BMI <30 and 23.8% had BMI ≥ 30. Patients with a family history of DM had higher mean BMI (26.93 kg/m^2), than those without family history (26.19 kg/m^2) – $P=0.01$. The weight gain during gestation was adequate in 41.4%, reduced in 29.9% and excessive in 28.7% of the patients. The prevalence of normal arterial blood pressure was 86.5%, hypertension worsened by pregnancy was 6.9% and pregnancy induced hypertension was 6.6%, the mean BMI in these three groups were 26.1, 30.51 and 29.33, respectively ($P<0.05$). There was statistical significant difference ($P<0.05$) between the two groups in these parameters: Insulin therapy 75.2% in Go *vs* 52.5% in Gno and its need was earlier in Go -28.83 wks *vs* Gno -30.97 wks; time of delivery 38.1 wks in Go vs 38.4 wks in Gno; caesarean section 49.8% in Go vs 35% in Gno; new- born weight 3324.8 g in Go vs 3167.9 g in Gno; macrosomic babies 8.3% in Go vs 4.4% in Gno. In the re-evaluation post-partum we found that higher BMI were related with severe degrees of carbohydrate intolerance: the mean BMI in the DM group was 29.53, in the IGT was 28.16, in the IFG was 26.99 and in the NGT was 26.55, $P<0.05$. We didn't find any difference in the re-evaluation between the women with adequate and excessive weight gain.
Conclusions
In GDM, obesity was found to be an increased risk for hypertension, earlier insulin need, earlier delivery, caesarean delivery, high baby weight and macrosomic babies. Pre-pregnancy BMI has a positive correlation with the development of carbohydrates intolerance. Thus we conclude that obesity in GDM is a risk factor for maternal and fetal outcomes, with the risk of early development in the mother of glucose intolerance.

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Sleep loss and metabolic response to breakfast

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Background
Sleep loss is increasingly common in the western world and has been shown to be associated with an increased risk of diabetes mellitus and obesity.
Objective
We hypothesised that short term sleep loss results in a diabetogenic metabolic status during carbohydrate challenge at a breakfast buffet.
Subjects and methods
Fifteen healthy, normal-weight young men were studied in randomized balanced order on the subsequent morning after (i) two nights with 8 h sleep and (ii) after two nights with only 4 h sleep in the second half of the night. Blood glucose and relevant hormones (insulin, C-peptide, and glucagon) were measured after awakening and during a standardised breakfast buffet. The study was approved by the ethics committee of the University of Lübeck and all subjects gave written informed consent.
Results
Circulating morning concentrations of any parameter were comparable between conditions ($P>0.30$ for all comparisons). There was no difference in carbohydrate composition of ingested breakfast between conditions ($P>0.35$). In response to breakfast blood glucose ($P<0.001$) as well as serum insulin ($P=0.02$) and C-peptide concentrations ($P=0.01$) raised to distinctly higher

concentrations in the 4 h than in the 8 h sleep condition. Also, plasma glucagon levels increased rapidly in response to breakfast with concentrations being constantly lower after 4 h than 8 h sleep ($P=0.03$).
Conclusion
Data indicate that short-term sleep loss markedly reduces glucose tolerance with the pattern of blood glucose and insulin response pointing to insulin resistance as the underlying mechanism. Thus results provide strong evidence for a causal link between sleep loss and the development of type 2 diabetes mellitus.

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Abdominal fat and atheromatosis risk factors in non-obese healthy subjects

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In the 1980s the concept, that some individuals with normal weight (Metabolically Obese, Normal-Weight) have disturbances of metabolism, related to obesity was proposed. It is known, that obesity promotes atheromatosis by various mechanism, inter alia due to excess of inflammatory markers. This problem however, was not investigated in non-obese subjects.
We analyzed anthropometric variables, body fat distribution by DXA and serum CRP, Il-6 and Il-18 in 431 healthy, non-obese Polish subjects: 232 women (age 32 ± 5.5; BMI 21.3 ± 2.7) and 199 men (age 30 ± 6; BMI 24.9 ± 2.9).
In our study abdominal fat volume was significantly correlated with CRP ($r=0.3$; $P<0.001$) in women, but there were no significant correlations between abdominal fat volume and serum Il-6 ($r=0.02$; $P=0.7$) or Il-18 ($r=0.07$; $P=0.3$). We demonstrated strong correlations between WHR and CRP ($r=0.2$; $P=0.004$), and Il-18 ($r=0.16$; $P=0.02$), but not with Il-6 ($r=0.005$; $P=0.9$).
Similar significant correlation between abdominal fat volume and CRP ($r=0.3$; $P<0.001$) was noticed in men. In this group also Il-18 ($r=0.16$; $P=0.03$) was strongly correlated with abdominal fat. On the contrary, there were no significant correlations between abdominal fat depot and serum levels of Il-6 ($r=-0.1$; $P=0.16$). Strong correlation between WHR and CRP ($r=0.2$; $P=0.002$), and Il-18 ($r=0.19$; $P=0.02$), but not Il-6 ($r=-0.05$; $P=0.5$) was seen.
In conclusion, not only in obese, but also in non-obese young healthy subjects visceral fat influences some inflammatory markers of atheromatosis: CRP in women, and CRP and Il-18 in men.

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The effect of fasting on substrate metabolism and growth hormone signaling

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Background
Fasting is associated with suppression of IGF-I production, which is likely to be the stimulator of GH secretion and the subsequent increase in lipolysis. The GH signalling events subserving this switch in the actions of GH have not been studied.
Aim
To assess whole body substrate metabolism and GH signalling proteins in skeletal muscle and fat in healthy human subjects in the postabsorptive state and after 38 h of fasting.
Design
In a randomized crossover design ten healthy non-obese young males were examined postabsorptively (14 h-fast) and after 38 h of fasting (38 h-fast). A bolus of GH was administered intravenously on each occasion followed by infusion of [3-3H]glucose. Muscle- and fat biopsies were taken one hour later. A hyperinsulinaemic euglyceamic clamp was performed after 2½ h. Intrahepatic lipid content (IHL) was assessed by ^{1}H- MR spectroscopy.
Results
Endogenous GH were higher during the 38 h-fast ($P<0.00$). Before and 2½ h after the GH bolus, significantly higher levels of FFA ($P<0.00$ vs $P<0.00$), glycerol ($P=0.02$ vs $P<0.00$) and 3-hydroxybutyrates (3-OHB) ($P<0.00$ vs $P<0.00$) were found after the 38 h-fast. The GH-induced increase in 3-OHB was significantly higher during the 38 h-fast ($P<0.00$). IHL increased significantly during fasting and correlated with circulating levels of ketone bodies. We found no significant difference between pSTAT5/STAT5 ratio (western blot), STAT5-DNA-complexes (EMSA), or IGF-I and SOCS3 mRNA levels when comparing the 14 h-fast and 38 h-fast. Hepatic and peripheral insulin sensitivity was significantly decreased during the 38 h-fast.
Conclusion
1) Prolonged fasting is associated with elevated GH levels, increased lipolysis and IHL, and hepatic and peripheral insulin resistance, 2) We could not detect fasting-induced alterations in GH signaling proteins in either muscle or fat, 3) The molecular mechanisms underlying the changes in the actions of GH during fasting remain to be characterized.

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Regulation of acylation stimulating protein by insulin in overweight and obese postmenopausal women: a MONET study

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Objective
Acylation Simulating Protein (ASP) has been shown to regulate lipid clearance and glucose uptake in adipose tissue. In vitro studies demonstrate that insulin stimulates ASP secretion from adipocytes. Individuals with obesity and/or metabolic disturbances (insulin resistance and type 2 diabetes) have increased plasma ASP, suggesting ASP resistance. Therefore, the present study evaluates whether ASP levels are influenced by the metabolic profile of overweight and obese postmenopausal women during a euglycemic/hyperinsulinemic clamp (EHC).
Materials and methods
The study population consisted of 76 overweight and obese sedentary postmenopausal women. We evaluated insulin sensitivity using the EHC, ASP levels, body composition (fat mass and visceral adipose tissue area), blood lipid profile, liver enzymes, maximal aerobic capacity (VO_{2peak}), resting metabolic rate and total energy expenditure using doubly labeled water.
Results
We observed wide inter-individual variations of ASP levels during the EHC. Therefore, subjects were divided into two groups based on a $>20\%$ change in ASP levels. Negative ASP Responders (NAR; $n=24$) showed, at least, a -20% decrease in ASP levels while Positive ASP Responders (PAR; $n=42$) displayed hormonal fluctuations superior to $+20\%$. Ten subjects had an ASP change of less then 20% and were excluded from the analysis. PAR women displayed a worse metabolic profile then NAR women: higher BMI, visceral adipose tissue, fasting insulin levels, lean body mass, and alanine aminotransferase (ALT), a marker of impaired liver function. After adjustment for BMI, only ALT remained significantly different while lean body mass ($P=0.08$) and visceral adipose tissue ($P=0.07$) remained marginally higher. In PAR and NAR subjects, fasting ASP levels correlated positively with albumin and VO_{2peak} and this association remained significant after adjustments for the effect of BMI. In addition, the % maximal change in ASP levels during the EHC was positively associated with aspartic acid aminotransferase (AST) and ALT.
Conclusion
Overall these results suggest that an elevated ASP response during the EHC is associated with metabolic disturbances in overweight and obese postmenopausal women.

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Prevalence of obesity and overweight in Iranian middle aged adults

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Introduction
High prevalence of obesity and overweight among middle aged adults is a major health problem. It is related to high prevalence of many chronic degenerative diseases in this high risk group. This study was conducted to determine the prevalence of obesity and overweight among young and middle aged Iranian adults.
Methods
This study was based on anthropometric data collected in the national household food consumption and nutritional status survey (2001–2003). Seven thousand and one fifty eight households were selected randomly. Height (to the nearest 0.1 cm) and weight (to the nearest 100 g) of 15636 adults aged 20–65 years were measured according to the standard protocols. BMI (kg/m^2) was calculated. Obesity and overweight were defined as BMI ≥ 30 and BMI 25–29.9 kg/m^2 respectively.
Results
Mean ($\pm$S.D.) and median of weight and height of young and middle aged adults were 67.1($\pm$13.7), 66.0 kg and 162.6($\pm$10), 162 cm and 68.5($\pm$13.0), 68.0 kg and 160.5($\pm$9.4), 160 cm respectively. Median BMI for two groups was 24.8 and 26.2 kg/m^2. Prevalence of obesity and overweight in young adults were (17%, 31.6%) and middle aged were (22.9%, 37.5%) respectively.
Conclusion
Prevalence of obesity and overweight especially in middle aged Iranian adults is similar to developed countries such as Canada and England. Obesity and overweight must be considered as a major risk factor for high prevalence of chronic diseases (coronary heart diseases, diabetes type 2) in Iranian middle aged adults.

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Determination of dimerisation domains of the human melanocortin 4 receptor

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The melanocortin 4 receptor (MC4R), a G protein coupled receptor, has a prominent role in hypothalamic weight regulation. The MC4R was recently shown to form receptor dimers. The cannabinoid 1 receptor (CB1R) is the nearest phylogenetic relative of MC4R but fails to interact with the MC4R in vitro. In this study we aim to investigate receptor domains that are necessary for MC4R interaction. Four different chimeric receptors of MC4R and CB1R were constructed. The generated constructs were transfected into COS-7 cells. Functional characterization included the determination of total expression, cell surface expression and dimerisation studies by a sandwich-ELISA approach as well as ligand binding and signal transduction properties. All constructs were investigated in comparison to MC4R homodimers. Exchanging of the transmembrane domain (TM) 1 and 2 (construct 1) or TM 4-7 (construct 2) resulted in a complete loss of expression. Total expression of constructs where the MC4R is exchanged with the following parts of the CB1R: TM4 alone (construct 3) or TM 3 to TM 4 (construct 4) could be determined but cell surface expression was reduced compared to the MC4R (85 and 58% respectively). No ligand binding for constructs could be determined therefore these constructs resulted in a total loss of function indicating the involvement of TM 4 and possibly TM 3 in ligand binding. Investigation of homodimerisation of construct 3 and 4 could demonstrate a strong interaction for construct 3 but no interaction of construct 4. For construct 3 also interaction of the MC4R could be observed. These findings point to a crucial role of TM3 and intracellular loop 2 for dimerisation of the human MC4R. Ongoing studies will closer narrow the domains of amino acids involved in interaction.

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Fluctuation of orexin, ghrelin and melatonin levels during daytime

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Introduction
Melatonin plays a key role in the circadian timing system. The question remains whether changes in endogenous melatonin may be associated with food intake. Orexin and ghrelin are involved in the regulation of energetic homeostasis. Therefore, we decided to obtain more detailed data on the circadian changes of orexin, ghrelin and melatonin for identification of the changes in these hormones to food intake and day-time, together with glycemic and C-peptide levels.
Methods
Five women (mean age 31.6 ± 2.8 years, mean BMI 23.2 ± 2.3 kg/m^2) in follicular phase of menstrual cycle was examined. The levels of orexin, ghrelin, melatonin, C-peptide and glucose were studied during a daily regimen (16 h) including standardized food intake. The diurnal profiles of the hormones and serum glucose were evaluated using ANOVA with Period and Subject as independent factors. The correlations between melatonin and the remaining parameters were assessed by Pearson's correlations and using a multiple stepwise backward regression model consisting of the time factor as a polynomial, and serum C-peptide and glucose. The study was approved by local Ethical Committe.
Results
The levels of blood glucose and C-peptide reflected periodic food intake being in a physiological range. A significant negative correlation between melatonin and C-peptide was found (Pearson's correlation, $r=-0.5525$, $P<0.0001$, $n=50$, partial correlation $r=-0.3532$, $P<0.02$, $n=50$). A borderline significant relationship between melatonin and blood glucose was detected (Pearson's correlation, $r=-0.4679$, $P<0.0006$, $n=50$). Ghrelin negatively correlated with C-peptide ($r=-0.356$, $P<0.02$). We found positive correlation between melatonin and ghrelin ($r=0.453$, $P<0.003$). Our results showed no significant correlation between orexin levels and other measured variables.
Conclusions
The negative relationship between melatonin and C-peptide as well as relatively rapid changes in melatonin levels permits speculation about food as one of the factors influencing daytime melatonin production.
The study was supported by grant: NR9055-4 IGA MZCR and GAUK.

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Metabolic syndrome and endothelial dysfunction in women with polycystic ovary syndrome – relationship with insulin sensitivity

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Polycystic ovary syndrome (PCOS) is a main cause of women infertility. There are data suggesting an increased risk for cardiovascular disease in PCOS. Insulin resistance might play a role in the pathogenesis of PCOS and its metabolic complications. The aim of the study was to asses the prevalence of The National Cholesterol Education Program (NCEP)-defined metabolic syndrome (MS) in women with PCOS in relation to insulin resistance and endothelial dysfunction – soluble E-selectin (sE-selectin) and soluble intercellular cell adhesion molecule-1 (sICAM-1).Euglycemic hyperinsulinemic clamp, serum sE-selectin, sICAM-1, and sex hormones were measured in 97 women with PCOS (33 lean and 64 overweight or obese) and 33 healthy women (22 lean and 11 overweight or obese). MS was present in 21 PCOS patients (21.6%). Both the lean and obese women with PCOS had lower insulin sensitivity ($P=0.03$ and $P=0.01$). The PCOS group had significantly higher serum concentrations of sICAM-1 and E-selectin than control group ($P=0.008$ and $P=0.01$, respectively). The comparison of the subgroups of patients with PCOS according to the presence of the MS revealed the significantly higher sICAM-1 ($P<0.001$) and E-selectin ($P<0.001$) concentration, lower insulin sensitivity ($P<0.001$), SHBG ($P=0.005$) and higher free androgen index (FAI) ($P=0.018$) in patients with PCOS and MS. Analysis of variance showed that together with the increase of the number of NCEP criteria in PCOS group, there was a significant decrease of insulin sensitivity, SHBG and an increase in sICAM-1 and E-selectin serum concentration and FAI ($P<0.001$). The significant inverse correlations between the insulin sensitivity and sICAM-1 ($r=-0.33$, $P=0.002$) and E selectin ($r=-0.33$, $P<0.0001$) were observed. Our study indicates that in young PCOS women, insulin resistance is associated with both classical and non-classical risk factors for cardiovascular disease.

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Diabetogenic effects of a continuous infusion of acylated ghrelin in normal and type 2 diabetic patients

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Background
Previous studies have shown that acylated ghrelin (AG) modulates insulin secretion and glucose disposal *in vitro* and after acute administration in humans. The aim of the present study was to evaluate postprandial glucose and insulin response following AG infusion in normal subjects and patients with type 2 diabetes.
Methods
Six normal subjects and 3 patients with type 2 diabetes were included in this study. AG (1 μg/kg per h) or saline were continuously administrated iv for 5 h and a standardized lunch of ~920 Kcal (50% carbohydrates, 30% lipids and 20% protein content) was served after 2 h of infusion. Blood samples were collected at times 0, 15, 30, 45, 60, 90, 120, 150 and 180 min in order to assay insulin levels and glycemia.
Results
Before meal, insulin levels (saline: 12.7±3.3 μIU/ml; AG: 13.1±6.6 μIU/ml) and glycemia (saline: 70.8±6.8 mg/dl; AG: 75.3±9.1) were not significantly different for both treatments. In postprandial conditions, AG infusion upregulated the glycemic peak value (153.2±22.6 mg/dl vs 110.0±9.3; $P=0.03$) while insulin concentrations were marginally increased (205.9±114.5 μIU/ml vs 96.3±17.2 μIU/ml; $P>0.05$) when compared with saline. Continuous AG administration induced a significantly increased of glycemic AUC (22 041±3758 mg/dl per min vs 15 273±834 mg/dl per min) while insulin AUC (24 959±13 310 μIU/ml per min vs 10 027±1933 μIU/ml per min; $P>0.05$) tended to be elevated compared to the one observed during saline administration. In addition, preliminary data suggest that AG infusion could also confer detrimental effects (increased insulin secretion and elevation of glycemia) in patients with early onset of type 2 diabetes.
Conclusion
The present study is the first to evaluate the influence of AG infusion in postprandial conditions both in normal subjects and patients with type 2 diabetes. Finally, these results strongly suggest that continuous administration of AG induces diabetogenic effects.

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Pentraxin 3 production in human visceral adipose tissue is associated with cardiovascular risk factors

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Pentraxin 3 (PTX3) is an acute phase protein expressed in the advanced atherosclerotic lesions and produced by endothelial cells, macrophages and, in rodents also by adipocytes under tumour necrosis factor α (TNFα) exposure. We investigated if PTX3 is expressed in human adipose tissue and is associated with cardiometabolic risk factors.
Methods
Samples of subcutaneous (SAT) and omental visceral (VAT) adipose tissue were obtained from 21 obese (BMI 38.8±4.48 kg/m^2, 37.4±8.15 years) and 10 normal weight subjects (BMI 23.8±1.69 kg/m^2, 43.7±11.07yr) who underwent abdominal surgery and had normal leucocyte count. Real-time PCR was used to quantify specific mRNA for PTX3, CD68 (macrophage marker), TNFα and adiponectin. Fresh adipose tissue was cultured and PTX3 measured in the medium. Serum insulin, glucose, HDL and LDL cholesterol, triglycerides, C- reactive protein (CRP), fibrinogen, adiponectin, TNFα and PTX3 were measured.
Results
PTX3 was expressed by adipocytes at similar levels in obese and normal weight subjects and in the two fat compartments. CD68 and PTX3 expression were correlated each other in SAT but not in VAT. In the whole group of subjects, after adjustment for age and sex, VAT PTX3 expression and release were correlated with VAT TNFα expression (r 0.537 and r 0.773, $P<0.01$ for both) and with LDL/HDL ratio (r −0.471, $P<0.01$ and r 0.773, $P<0.001$). VAT-PTX3 expression was also correlated with BMI (r 0.365, $P<0.05$), triglycerides (r 0.420, $P<0.05$), CRP (r 0.475, $P<0.05$), fibrinogen (r 0.446, $P<0.05$) and adiponectin (r −0.390, $P<0.05$). In the multivariate analysis with VAT-PTX3 RNA levels as dependent variable, LDL/HDL ratio and fibrinogen remained independently associated with VAT-PTX3 expression (β 0.521, $P<0.01$ and β 0.616, $P<0.01$). These associations were not seen within SAT.
Conclusions
Human adipose tissue expresses and releases PTX3 possibly under TNFα control. VAT production of PTX3 seems to contribute to the mechanisms underlying the development of atherosclerosis.

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Insulin and glucose regulates omentin-1, a novel adipokine: relation to women with the polycyastic ovary synfrome

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Polycystic ovary syndrome (PCOS) is associated with insulin resistance and obesity. Recent studies have shown that plasma omentin-1 levels decrease with obesity. Currently, no data exists on the relative expression and regulation of omentin-1 in adipose tissue (AT) of PCOS women.
Objectives
To assess mRNA and protein levels of omentin-1 in omental (om) AT of PCOS women and matched controls, including circulating omentin-1. *Ex vivo* and *in vivo* regulation of AT omentin-1 was also studied.
Research design and methods
Real-time RT-PCR and western blotting were used to assess mRNA and protein expression of omentin-1. Plasma Omentin-1 was measured by ELISA. The effects of D-glucose, insulin, gonadal and adrenal steroids on AT omentin-1 were analysed *ex vivo*. The *in vivo* effects of insulin (hyperinsulinemia) on omentin-1 levels were also assessed by a prolonged insulin-glucose infusion.
Results
In addition to decreased plasma omentin-1 levels in PCOS women ($P<0.05$), compared to controls, there was significantly lower levels of omentin-1 mRNA ($P<0.01$) and protein ($P<0.05$) in om AT of PCOS women ($P<0.01$). Furthermore, in om AT explants, insulin and glucose significantly dose-dependently decreased omentin-1 mRNA expression, protein levels and secretion into conditioned media ($P<0.05$, $P<0.01$). Also, hyperinsulinemic induction in healthy subjects significantly reduced plasma omentin-1 levels ($P<0.01$).
Conclusions
Our novel findings reveal that omentin-1 is down regulated by insulin and glucose. These may in part explain the decreased omentin-1 levels observed in our overweight PCOS women.

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Postnatal determination of ghrelin secretion levels and sexual dimorfism in the rat

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Ghrelin is a pleiotropic hormone characterized mainly by its strong orexigenic action and its role as GH secretagogue.
Age-related level variation of this hormone has been previously shown in different studies centred in mARN expression and in its plasma levels; however there is no data about its secretion directly from the stomach although this is the main ghrelin producing organ.

Objectives
To determine the levels of ghrelin in the postnatal development and its possible sexual dimorfism.
Methods
Real Time PCR, RIA, Organ culture, Surgery.
Experimental groups
Male and female rats from 1 to 24 weeks.
Rats subjected to ovariectomy and orquidectomy.
Results
Our results showed a diminution of ghrelin plasma levels in the second week; levels were normalized in the following weeks. In relation to ghrelin secretion from the stomach, we found a great decrease of secretion in the fourth week and a peak in the sixth week of development.
Gastric ghrelin mARN levels increased along time in a non significant manner. Gonadectomy did not change plasma ghrelin levels although it did affect gastric ghrelin levels.
Conclusions
The weaning and the puberty in the rats regulate gastric ghrelin secretion during postnatal development. Sexual hormones can influence the secretion of ghrelin.

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The effect of polyunsaturated fatty acids enriched eggs on lipid profile and inflammatory markers

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The correction of a subtle nutritional deficiency that may reduce the risk of a future chronic disease is indeed a challenge. One of the most intriguing current and future impacts on public health may come from a greater intake of omega-3 fatty acids such as α-linolenic acid (ALA).
Objective
We investigated the effect of an increased amount of dietary α-linolenic acid (ALA) from enriched eggs on the lipid profile and inflammatory markers in healthy volunteers.
Subjects and methods
Sixty-two subjects were voluntary enrolled after they gave their informed consent. They were randomly assigned in either control or omega group. Control group consumed normal eggs while omega group consumed eggs enriched in omega 3 fatty acids. The content of ALA in omega 3 eggs was 5 times greater than that of control eggs. During the study, all subjects maintained their habitual diets except that egg consumption. Each subject had to consume 6 eggs a week during a 6-week period.
Blood samples were collected at day 0 (baseline) and at the end of the study.
Triglycerides, cholesterol, HDL, LDL cholesterol, ApoA, ApoB, CRP and fibrinogen were measured in serum samples.
Results
We compared the measured values of the biochemical parameters at baseline and after egg consumption both in control and omega group. Triglycerides were significantly reduced in omega group ($P=0.002$) after omega-3 enriched eggs consumption but not in control group. Fibrinogen level was significantly decreased ($P<0.001$) by omega-3 enriched eggs consumption whereas in control group there were no significant changes. No significantly changes were found in the other parameters of the lipid profile or CRP.
Conclusion
Omega-3 dietary supplementation decreases trygliceride and fibrinogen level. Omega-3 enriched eggs can be considered as functional food with beneficial effects on human health.

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Carbohydrate and lipid oxidation in relation to serum adiponectin concentration in women with anorexia nervosa

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Anorexia nervosa (AN) is an eating disorder, with the significant loss of the adipose tissue. Lack of subcutaneous adipose tissue observed in lipodystrophies is accompanied by insulin resistance. The crucial step in the development of insulin resistance is an impaired increase in carbohydrate oxidation and decrease in lipid oxidation in response to insulin. The aim of the present study was to estimate carbohydrate and lipid oxidation in relation to serum adiponectin concentration in women with AN.
We examined 16 women with AN, 15 lean control women and 15 obese women. Euglycemic hyperinsulinemic clamp, indirect calorimetry in baseline state and during the last 30 min of the clamp and the measurements of serum adiponectin concentration were performed.
Insulin sensitivity was decreased in obese women in comparison to AN ($P=0.0045$) and controls ($P=0.015$), whereas it was not different between AN and controls. Serum adiponectin was higher in AN women in comparison to other groups (control, $P=0.015$; obese, $P=0.038$) and in control in comparison to the obese women ($P=0.047$). Women with AN had preserved carbohydrate and lipid oxidation, and non-oxidative glucose metabolism during hyperinsulinemia. All these parameters were not significantly differ from control group, although lipid oxidation in the hyperinsulinemic state tended to be lower in AN ($P=0.066$). Obese women had lower carbohydrate oxidation during the clamp in comparison to AN ($P=0.022$) and controls ($P=0.01$) and lower non-oxidative glucose metabolism and higher lipid oxidation during the clamp in comparison to AN ($P=0.016$ and $P=0.038$, respectively). Serum adiponectin was related to lipid oxidation during hyperinsulinemic state ($r=-0.38$, $P=0.039$).
Women with AN had normal insulin sensitivity due to the preserved response to insulin of substrate oxidation and non-oxidative glucose metabolism. Adiponectin is related to insulin sensitivity through its association with the suppression of lipid oxidation during hyperinsulinemia.

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Molecular insights in dysfunctions of the human melanocortin-4-receptor (MC4R) caused by mutations in the third transmembrane domain (TM3) and the second intracellular loop

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Mutations in the hypothalamic expressed MC4R gene are the most frequent cause of monogenetic obesity. This $G\alpha_s$-Protein coupled receptor (GPCR) is activated by endogenous ligands α- and β-MSH and is inhibited by the only known endogenous inverse agonist and antagonist Agouti related peptide (AgRP). Naturally occurring mutations help to understand activation mechanisms of the human MC4R.
We previously described a constitutively activating MC4R mutation (H158R) found heterozygously in a normal weight proband. This mutation is located in the second intracellular loop and causes a six times higher basal activity, a higher maximal stimulation and a slightly decreased EC_{50} after stimulation with MSH-ligands when compared to the WT-MC4R. The increased basal activity can be dose dependently reduced by stimulation with the inverse agonist AgRP. When the WT-MC4R is stimulated with α-MSH in presence of increasing AgRP concentrations, an increase of EC_{50} is observed, which can not be seen for the H158R mutant, explaining the normal phenotype of the mutation carrier. The histidine at position 158 is conserved in the MC4Rs of 70 species and also in all 5 human melanocortin receptors but not generally in GPCRs. Exchanging the histidine to further amino acids by site directed mutagenesis decipher the crucial role of histidine 158 for receptor silencing. Additionally, in contrast to activating mutation H158R we describe three inactivating mutations at serine residues located in the third transmembrane domain, which were found in obese patients. S136F causes a loss of function although properly expressed on the cell surface and shows a dominant negative effect when co-transfected transiently with WT-MC4R. S127L is a partial loss of function mutation with normal cell surface expression pattern. Molecular details of modifications on the MC4R structure and signaling properties caused by these mutants are evaluated using molecular modelling driven side chain substitutions. Furthermore, S139R resulted in a complete loss of function and impaired membrane trafficking. Exchanging S139 to isoleucine partially rescued these effects. In a computer-generated receptor model, substitution of S139 with the bulky and charged arginine disrupts the conserved interaction of serine 85 in TM2 with tryptophan 174 in TM4 and forms a new constraining interaction to TM4 thus leading to inadequate folding of the

receptor. These new findings reveal deeper insight into the intramolecular mechanism of activity regulation at the human MC4R.

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Ghrelin gene polymorphisms in Prader Willi Syndrome

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Introduction
Prader Willi Syndrome (PWS) is a genetic syndrome characterized by hyperphagia, morbid obesity, and many other endocrine alterations. PWS subjects present higher ghrelin levels. The cause of this increase as well as the modulation of ghrelin secretion at fasting and feeding in relation to other metabolic parameters in PWS is largely unknown. It has also been demonstrated that many ghrelin gene (GHRL) polymorphisms are associated with obesity, type 2 diabetes, and hypertension. Despite these data, the physiologic role of regulation of GHRL expression in normal condition as well as in PWS has not been fully clarified so far.
Subjects and methods
To this aim, mutational analysis of the total GHRL in unrelated 90 normal-weighted (CCNW), 81 obese (OB) healthy young subjects, and 34 children or adult PWS were performed. We also evaluated lipid metabolism, fasting glucose in children and glucose tolerance in adult PWS. Results. The pre-pro GHRL variants and a SNP of the promoter region (rs26802) showed similar allele frequency in CCNW, OB, and PWS. All PWS were carriers for a second common polymorphism of SNP in the promoter region (rs27647) ($P<0.002$ and $P<0.003$ in comparison to CCNP +OB, and CCNP or OB, respectively). No significant differences in rs27647 were recorded between CCNP and OB. Moreover, the rs26802 genotype (A>C) distribution was different between euglycemic and diabetic adult PWS ($P<0.02$). No other associations were found between the other polymorphisms and the metabolic parameters in PWS. Conclusions. These data report for the first time that polymorphisms in the 5′ flanking region of GHRL seem differently distributed in PWS compared to normal population and that they may be associated to the development of a diabetic phenotype in adult PWS.

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Retinol-binding protein 4 is independently associated with insulin resistance in PCOS women

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Objective
Adiposity, insulin resistance, and hyperandrogenism are features of the polycystic ovary syndrome (PCOS). Retinol-binding protein 4 (RBP4) secreted from adipose and liver tissue has been linked to insulin resistance. We here address the impact of RBP4 on insulin resistance in PCOS and its usability to identify women with metabolic syndrome or impaired glucose metabolism (IGT or diabetes).
Design
RBP4 was determined in plasma of 115 PCOS women. Associations with insulin resistance, body composition, and hyperandrogenemia were investigated by correlation and multiple linear regression analyses in 110 non-diabetics. Receiver operating characteristic (ROC) curve analysis was used to evaluate RBP4's usability for identifying impaired glucose metabolism or impaired glucose metabolism.
Results
RBP4 increased over tertiles of insulin resistance ($P=0.009$). RBP4 correlated with insulin resistance (HOMA%S ($R=-0.286$, $P=0.002$)), WHR ($R=0.233$, $P=0.034$), and DEXA-lean body mass ($R=0.282$, $P=0.016$) but not with BMI, DEXA-total or -trunk fat mass, hsCRP, free testosterone, DHEAS, androstendione and 17β-estradiol. Adjusted for age, BMI, smoking and IGT the association between RBP4 and HOMA%S remained significant ($P=0.032$). However, RBP4 explained only 4.6% of the variation of HOMA%S. RBP4 was higher in metabolic syndrome and in impaired glucose metabolism, but its usability to identify women with metabolic syndrome or impaired glucose metabolism was low (AUCs 0.631, $P=0.041$ or 0.660, $P=0.016$).
Conclusions
In PCOS RBP4 has a small independent impact on insulin resistance. It is neither correlated to hyperandrogenemia, 17β-estradiol or other adrenal steroids nor to markers of adiposity in general. Furthermore, RBP4 does not appear suitable for screening metabolic syndrome or impaired glucose metabolism.

P541

Glucagon suppression of total but not of acylated ghrelin is preserved in obesity: the impact on appetite control

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Objective
The mechanisms underlying the well known glucagon-induced satiety effect are unclear. As we showed recently, the glucagon-induced reduction in total ghrelin, that might be responsible for this effect, is exerted at hypothalamus–pituitary level. The aim of the present study was to further evaluate glucagon's suppressive effect on both total and acylated-ghrelin in obesity with respect to its role in appetite control.
Methods
Prospectively, we studied the endocrine and metabolic responses to intramuscular glucagon administration in 14 lean (6 males; BMI 21.6 ± 0.6 kg/m^2) and 12 obese healthy subjects (6 males; BMI 33.9 ± 1.6 kg/m^2). All subjects were proved to have an intact pituitary function.
Results
Age, fasting glucose, glucagon and acylated-ghrelin concentrations were comparable between both groups. Fasting insulin was significantly higher and baseline total-ghrelin was significantly lower in obese than in lean subjects. Total-ghrelin significantly decreased after glucagon administration in both obese (mean ± S.E.M.: 706 ± 54 vs 618 ± 47 pg/ml, $P<0.01$) and lean subjects (1181 ± 133 vs 1023 ± 102, $P<0.01$). Acylated-ghrelin did not change significantly in obese, whereas a significant decrease occurred in lean subjects (306 ± 58 vs 209 ± 43 pg/ml, $P<0.05$). However, hunger scores significantly decreased in both groups: lean (3.2 ± 0.3 vs 2.3 ± 0.2, $P<0.05$) and obese subjects (4.1 ± 0.5 vs 2.9 ± 0.4, $P<0.05$).
Conclusions
We show that glucagon significantly decreases total- and acylated-ghrelin in lean healthy subjects but fails to affect acylated-ghrelin in obese individuals. The glucagon-induced satiety effect remained intact indicating the role of changes in total rather than in acylated-ghrelin in mediating this effect.

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The sirtuin activating compound resveratrol decreases GH transcription

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Caloric restriction is the best known experimental procedure that extends lifespan and resistance to aging-related diseases. Part of these actions was found to be mediated by the NAD-dependent histone deacetylase Sir2/Sirt1. Resveratrol is a polyphenol found in the skin of grapes, which was identified as a Sirt1 activator and subsequently proposed as a caloric restriction mimetic. The aim of the present study was to determine how resveratrol affects the GH axis, which is central in the physiology of aging. Treating rat anterior pituitary cells in primary cell culture

and GH3 GH-producing cells with resveratrol decreased GH secretion. GH promoter is regulated by the cAMP/PKA pathway through CREB-binding protein (CBP) bound to Pit1. Treatment with resveratrol decreased the binding of acetyl-histone 3 and Pit1 on the endogenous rat GH promoter. Furthermore Pit1 gene expression was found to be reduced in resveratrol treated GH3 cells. In rat cells Pit1 is under the control of itself and CREB. Resveratrol treatment decreased CREB acetylation and the specific Sirt1 activator NAD decreased CREB transcriptional activity. CREB is mainly acetylated by the histone acetyltransferase CBP. Sirt1 immunoprecipitated with CBP and resveratrol decreased CBP acetylation, an effect that results in diminishing its acetyltransferase activity. Interestingly, resveratrol also decreased CREB phosphorylation at Ser133. CREB is usually phosphorylated by the cAMP/PKA pathway. However, resveratrol treatment did not affect cAMP production. Other kinases, such as, Akt were also found to phosphorylate CREB. Indeed resveratrol treatment decreased pAkt-Ser473 levels in a mechanism involving mTOR but not PDK1. Altogether these data demonstrate that resveratrol decreases GH synthesis, similar to the situation observed in caloric restricted rodents, and provide a mechanism linking the lifespan regulator Sirt1 with the GH axis.

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Glucose-dependent insulinotropic polypeptide (GIP) modulates inflammatory markers and regulates cell proliferation in subcutaneous adipose tissue

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Background
Glucose-dependent insulinotropic polypeptide (GIP) is a gastrointestinal hormone that is secreted in response to food intake and modulates β-cell function. Remarkably, GIP looses its insulinomimetic effect in the presence of chronic hyperglycaemia and thus appears to be ineffective in poorly controlled T2DM at least with regard to insulin release. The mechanism by which this occurs is not clear and it is not known.
Objectives
To analyse the effect of GIP-Infusions on changes in adipose tissue gene expression at different blood-glucose levels in obese men and also detect different biomarkers and hormone interactions caused by GIP.
Setting and participants
Seventeen healthy overweight men (BMI: 28–40 kg/m^2; age: 30-65 years) underwent a single-blind intervention with euglycaemic or hyperglycaemic clamps in combination with GIP or saline infusions. Each solution was applied at physiological concentrations for four hours. Before and after the infusions biopsies from subcutaneous adipose tissue were taken. We isolated total RNA from all fat biopsies and hybridized the RNA to Agilent Whole Human Genome Microarrays (total 100 arrays). The results were verified by RT-PCR and amended by cell culture experiments.
Results
We identified several genes being involved in inflammatory and proliferative cell signalling. Under euglycaemic hyperinsulinaemic clamp conditions in combinations with a GIP-Infusion we find a significant upregulation of chemokine ligand 2 (CCL-2/MCP-1), interleukin Iβ (IL-1β), fibroblast growth factor receptor 1 (FGFR-1) and oncostatin M (OSM). We also see an activation of cell proliferation by wnt signalling in adipose tissue after exposure with GIP mediated by cyclin D1, frizzeled homolog 4, frizzeled homolog 10 (FZD 4/10) and several zinc finger proteins like ZNF 397, and ZNF 658.
Conclusion
GIP may play a role in cell proliferation and inflammation in adipose tissue of obese men. Dependent from blood glucose levels GIP also effects lipid and glucose metabolism in adipose tissue.

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Serum soluble E-selectin concentration in relation to insulin resistance and metabolic inflexibility in obese women

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Markers of endothelial dysfunction, including soluble E-selectin (sE-selectin) are related to insulin resistance and are predictors of type 2 diabetes. Insulin resistance is associated with metabolic inflexibility, i.e. an impaired stimulation of carbohydrate oxidation and inhibition of lipid oxidation in insulin-stimulated conditions. The aim of the present study was to estimate the relationships of serum sE-selectin concentration with carbohydrate and lipid oxidation in lean and obese women. The study group consisted of 80 apparently healthy women: 33 lean and 47 with overweight or obesity. Euglycemic hyperinsulinemic clamp and indirect calorimetry in the baseline state and during the last 30 minutes of the clamp were performed. Obese women had lower insulin sensitivity ($P=0.0022$) and higher serum sE-selectin concentration ($P=0.034$). In the obese group, carbohydrate oxidation was increased in baseline state ($P=0.019$) and decreased during hyperinsulinemia ($P=0.048$) and lipid oxidation was higher during hyperinsulinemia ($P=0.0012$). Obese women had also lower increase in carbohydrate oxidation ($P=0.0012$) and lower decrease in lipid oxidation ($P=0.034$) during the clamp. Serum sE-selectin was negatively related to insulin sensitivity ($r=-0.27$, $P=0.015$). Higher serum sE-selectin concentration was associated with a lower increase in carbohydrate oxidation ($r=-0.41$, $P<0.001$) and a lower decrease in lipid oxidation ($r=0.29$, $P=0.008$) during the clamp. Serum sE-selectin was also inversely related to the direct measure of metabolic flexibility, i.e. to an increase in respiratory quotient in response to insulin ($r=-0.36$, $P=0.001$). Our data show that serum sE-selectin concentration is associated with both carbohydrate and lipid oxidation. Higher serum sE-selectin concentration might be linked to metabolic inflexibility of obesity and insulin resistance.

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The volume of left hepatic lobe as metabolic marker, correlates with weight and not with height, both in obese or non-obese women and men

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Introduction
Left hepatic lobe volume (LHLV) has been associated with hyperinsulinemia and insulin resistance in obese women.
Aim
To study the correlation between LHLV and body weight, regardless the weight and gender of the patient.
Subjects and methods
The left hepatic lobe was evaluated with an echographic probe of 3.5 MHz. The LHLV (cm^3) was calculated with the formula: antero-posterior diameter (length)×longitudinal length (right-left)×sagital diameter (length up-down)×0.52. The study group consisted of 648 women, aged between 6 and 87 years and 219 men, aged between 5 and 83 years. None of them had a known hepatic disease. Linear correlation was calculated between body mass index (BMI) and LHLV. Statistical analysis consisted of Student and Pearson test.
Results
LHLV values are presented in the following table. Differences between LHLV in non-obese versus obese women and men are highly statistically significant ($P<0.001$). Linear correlation between LHLV and BMI in all women was statistically significant ($r=0.64$, $P<0.001$), as well as in all men ($r=0.62$, $P<0.001$). We found no correlation between LHLV and height in both sexes ($r=-0.14$, resp. $r=0.13$).

	LHLV (cm^3)			LHLV (cm^3)	
Women	Average	SD	Men	Average	S.D.
All	173.9	67.77	All	203.77	91.36
Non-obese	153.75	49.99	Non-obese	179.00	66.42
Obese	236.93	77.58	Obese	281.32	113.60

Conclusions
LHLV is dependent on BMI and not on the patient height: the higher the body fat content is, the larger the left hepatic lobe is in both men and women. The quantitative estimation of LHLV may be a useful surrogate marker in the study of obesity related insulin resistance.

P546

Decreased NK cell functions from obese F344 rats can be altered after transfusion in lean littermates

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Leptin acts not only as an anorexigenic hormone but is also involved in the regulation of cell-mediated immunity. However, since the impact of leptin on NK cells is currently elusive, we investigated the body weight-dependent leptin effects on NK cells numbers and function.
In a first set of experiments leptin and MADB106 tumor cells were injected intravenously in male lean and diet-induced obese Lewis and F344 rats. In a second set of experiments an *in vivo* NK cell depletion with consecutive cross-over re-transfusion of NK cells in F344 rats was performed. Blood NK cell numbers were determined in blood and spleen by FACS and immunohistochemistry and the activity of NK cells was measured by chromium release assay. The Ob-R expression on NK cells was analyzed by confocal laser scanning and qRT-PCR. Intracellular signaling cascades downstream of Ob-R were evaluated by western blotting. Leptin application resulted in increased NK cell cytoxicity in lean rats but failed to activate NK cells from obese rats. We found Ob-R to be expressed on NK cells and qRT-PCR showed significantly higher Ob-Rb mRNA levels in NK cells from obese rats compared to lean littermates. In contrast, post receptor signaling was altered in obese animals with significantly lower activation of post-receptor signaling components (JAK-2p, PKBpT308, AMPKα-pT172) upon the leptin challenge. Results of the cross-over transfusion of NK cells impressively showed a time-dependent host-specific distribution and activation of NK cells.
The results for the first time demonstrate milieu-specific altered NK cell functions in obese animals and the reversibility of these changes after transfusion in normal weight littermates. The data have important implications for the influence of weight gain and loss on immune cell numbers and functions.

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Central resistin regulates both hypothalamic and peripheral lipid metabolism in a nutritional dependent fashion

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Current evidence suggests that the adipocyte-derived hormone resistin (RSTN) regulates both feeding and peripheral metabolism through unclear hypothalamic-mediated mechanisms. Here, we demonstrate by the first time, that the anorectic effect of RSTN is associated to specific changes in the expression of neuropeptides in the arcuate nucleus of the hypothalamus (ARC), namely AgRP, NPY and CART. Very interestingly, RSTN also exerts a deep, nutritional-dependent inhibitory effect on hypothalamic fatty acid metabolism, by increasing the phosphorylation levels of both AMP-activated protein kinase (AMPK) and its downstream target acetyl-CoA carboxylase (ACC), as well as decreasing the expression of fatty acid synthase (FAS), specifically in the ventromedial nucleus of the hypothalamus (VMH). In addition, we also demonstrate that chronic RSTN injection markedly reduces body weight and induces major changes in peripheral *de novo* lipogenesis in a tissue-specific and nutritional dependent fashion. Thus, in fed conditions central RSTN stimulates fatty acid synthesis in liver while in fast condition does so in white adipose tissue (WAT). Overall, our results indicate that hypothalamic actions of RSTN are a physiological mechanism controlling feeding and peripheral lipid metabolism and also that hepatic RSTN-induced insulin resistance may be mediated by central activation of lipogenesis *de novo* in liver.

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Changes in physical activity and changes in body weight: a longitudinal assessment in the SUN dynamic cohort

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Objective
To evaluate the associations between baseline leisure-time physical activity (LTPA) or changes in LTPA during follow-up on long-term weight changes, and to investigate the effects of physical activity in participants with baseline BMI $<$ 25 kg/m^2 and baseline BMI $\geq$ 25 kg/m^2.
Material and methods
Data of the prospective dynamic cohort (SUN cohort) were used. In total 12 117 participants, all of them university graduates, were followed-up for an average time of 27 months. Baseline LTPA was assessed with a previously validated questionnaire. We used self-reported data of body mass index (BMI), and a semiquantitative food-frequency questionnaire, both had been validated. The study was approved by the local Ethical Committee.
Results
After adjusting for age, smoking status, total energy intake, snacking, hours sitting down, alcohol intake, total fiber intake, the consumption of sugar sweetened beverages and fast food, participants who decreased their LTPA during follow-up experienced a significant ($P<0.001$) increase in body weight: ($+0.70$ kg for men, $+0.64$ kg for women). Participants who increased their LTPA during follow-up experienced a significant ($P<0.001$) reduction in body weight: (-0.66 kg for men, -0.39 kg for women). This inverse association between changes in LTPA and weight change was stronger for participants with a baseline BMI $\geq$ 25 kg/m^2 ($P=0.005$ for interaction in males and $P=0.01$ for interaction in females).
Conclusion
Longitudinal changes in LTPA during follow-up are inversely associated with changes in body weight, particularly in participants with initial BMI $\geq$ 25 kg/m^2. This association is apparently stronger than that between baseline LTPA and weight change.

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The relationship between alteration in plasma inflammation, metabolic biomarkers and severe steatosis in a group of morbidly obese patients

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Aim
The aim of the study was to evaluate the relation between anthropometric measurements, plasma biomarkers of inflammation, metabolic and adipokines pattern and the presence of severe steatosis, in a group of patients with morbid obesity.
Materials and methods
We evaluated a group of 40 patients (25 F, 15 M) with morbid obesity and indication for bariatric surgery. Anthropometric measurements (waist circumference, BMI determination) were performed and the presence and the degree of hepatic steatosis, right lobe diameter (measured by abdominal ultrasound), markers of inflammation (hs C reactive protein, fibrinogen, IL6) , insulin resistance (homeostasis model assessment-HOMA) and adiponectin level (ELISA method) were evaluated. According to US results, the patients were divided in a group with severe steatosis (17 patients) and a group with apparently normal structure or lesser degrees of steatosis (13 patients).
Results
Obese patients with severe steatosis had significantly higher waist circumference (137.7 vs 112.42 mm, $P<0.01$), liver right lobe diameter (193.8 vs 153.9 mm, $P<0.001$), HOMA (9.32 vs 4.07, $P<0.01$) and lower levels of adiponectin (14.15 vs 20.40 ng/ml, $P<0.05$) than those without severe steatosis, independently of the BMI. In all patients, right lobe transverse diameter was positively correlated with the level of plasma CRP ($r=0.535$, $P<0.05$), triglycerides ($r=0.4$, $P<0.05$), liver enzymes and GGT level. Patients with severe steatosis showed a higher prevalence of metabolic syndrome (ATP III criteria – 63% vs 50%, $P<0.05$).

Conclusions
These results show that severe steatosis is closely related with a modified plasma lipid and pro-inflammatory profile, as well as with markers of insulin resistance (HOMA). Adiponectin levels were significantly lower in patients with severe steatosis than in patients with lesser degrees of fatty liver. Further studies are needed in order to establish a direct causality effect between this alterations and liver changes in severely obese patients.

P550

Endocrine and metabolic alterations in narcoleptic patients as result of orexin-A reduction

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Aims
Narcolepsy with cataplexy (NC) is a rare, chronic disease, characterized by excessive daytime sleepiness, cataplexy, disrupted nocturnal sleep and manifestations of abnormal REM (Rapid Eye Movement) sleep. Human NC is probably caused by environmental and genetic factors, leading to dysfunctions in hypothalamic orexigenic neurons. Recent studies showed that NC was associated with an increase in Body Mass Index, despite a reduction in food intake. In the current study we investigated hormonal and metabolic patterns in patients with NC to determine whether an association between NC and hormonal and metabolic disorders may occur.
Methods
This was a case–control study approved by local Ethical Committee. We enrolled 23 patients with NC and two groups of control subjects, matched for age and BMI: in the first group we included 21 patients with other sleep disorders, while the second group composed by 19 patients was drawn from general population.
Results
In patients with NC we found a decrease in CSF orexin–A (NC = 13 pg/ml, controls = 109 pg/ml; $P = 0$), and a statistical significant increase in BMI and waist circumference; in the same patients an increase in plasma leptin levels, total and LDL cholesterol, and oestradiol, and a reduction in HDL cholesterol and plasma cortisol were also detected. These alterations, however, were detected only in male group. Our results confirm that NC is a disease due to low CSF orexin–A levels, and it is associated not only with metabolic disorders, such as perturbations in lipid profile, leptin secretion and body fat distribution, but also with endocrine alterations, especially regarding cortisol and estradiol secretion.
Conclusions
Studies on narcolepsy may provide interesting clues about the peripheral role of orexin-A in modulating hormones and metabolic processes.

P551

Prevalence of non alcoholic steatohepatitis in diabetic patients with morbid obesity

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Non alcoholic steatohepatitis (EHNA) is diagnosed by hepatic biopsy. Its etiology is uncertain but obesity is present in 69–100% of all cases and type 2 diabetes mellitus (DM) in about 34–75%.
Objectives
To study the prevalence of EHNA in morbidly obese patients and determine if there is a difference between the prevalence in patients with and without carbohydrate metabolism disorders.
Patients and methods
Prospective study of 120 females and 32 males with morbid obesity (IMC: 51.1 + 8.9 kg/m^2). The mean age was 42.4 + 9.3 years. Viral hepatitis was discarded by serological studies and toxic abuse with complete anamnesis. When the presence of DM was unknown an oral glucose tolerance test (OGTT) was done. During bariatric surgery hepatic biopsy was practised for pathological study; informed consent was obtained prior to the procedure. The data was analysed statistically by χ^2 test.
Results
Forty-eight patients (31.5%) presented type 2 DM, 32.4% of the cases were diagnosed by OGTT. The results of the pathological study of the hepatic tissue are:

Parameter	Normal	Steatosis	Steatohepatitis	Total
Normoglycemia	9.8%	33.6%	12.6%	56%
Impaired glucose tolerance	0.7%	5.6%	6.2%	12.5%
DM	3.5%	18.2%	9.8%	31.5%
Total	14%	57.4%	28.6%	

In diabetic patients steatosis was present with a frequency of 57.7% and EHNA of 31.1%. No significant differences were observed between the prevalence of EHNA and steatosis in patients with diabetes and those with normoglycemia.
Conclusions
Steatosis is the most frequent finding in diabetic morbidly obese patients followed by EHNA. The prevalence of EHNA in diabetic patients with morbid obesity is high. No differences were observed between the prevalence of EHNA in patients with DM and those withouth disorders of the carbohydrate metabolism. These results strongly suggest that obesity plays a more important role than DM in the etiology of the EHNA.

P552

Dual effect of the adapter Grb14 on insulin action in primary mouse hepatocytes

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Obesity and type 2 diabetes are expanding rapidly and become a wide world health issue. These metabolic diseases are tightly associated with an insulin resistance state. Among insulin target tissues, liver plays a central role in the regulation of glucose homeostasis.
Insulin action is initiated by its binding to its receptors (IR) which, once activated, phosphorylate intracellular substrates and lead to the activation of transduction pathways implicating the kinase Akt and the transcription factor SREBP-1c. The molecular adapter Grb14, which is highly expressed in the liver, binds to the insulin-stimulated IR and inhibits its tyrosine kinase activity. However, the physiological role of Grb14 in liver metabolism was unexplored. In this study, we used RNA interference to investigate the consequences of Grb14 decrease on insulin-regulated intracellular signaling and on glucose and lipid metabolism in mouse primary cultured hepatocytes.
In Grb14-depleted hepatocytes, insulin-induced phosphorylation of Akt, and of its substrates GSK3 and Foxo1, were increased. These effects on insulin signaling are in agreement with the selective inhibitory effect of Grb14 on the receptor kinase. However, the metabolic and genic effects of insulin were differentially regulated after Grb14 down-regulation. Indeed, the insulin-mediated inhibition of hepatic glucose production and gluconeogenic gene expression was preserved. Surprisingly, despite the improved Akt pathway, the induction by insulin of SREBP-1c maturation was totally blunted. As a result, in the absence of Grb14, glycogen synthesis as well as glycolytic and lipogenic gene expression were not responsive to the stimulatory effect of insulin. This study provides evidence that Grb14 exerts a dual role on the regulation by insulin of hepatic metabolism: it inhibits IR catalytic activity, and acts also at a more distal step, i.e. SREBP-1c maturation, which effect is predominant under short-term inhibition of Grb14 expression.

P553

Consequences of the modification of Grb14 expression level on adipocyte differentiation and metabolism

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Grb14 is a molecular adaptor that inhibits insulin signaling by interacting with the activated insulin receptor and inhibiting its tyrosine kinase activity. In various models of insulin resistance, including obese mice and type 2 diabetes patients, an inverse correlation was reported between Grb14 expression in adipose tissue and insulin sensitivity. To elucidate Grb14 function in this specific tissue, we studied the impact of modifications of Grb14 level of expression on adipocyte metabolism and differentiation.
We established a 3T3-L1 cell line with an inducible over expression of Grb14 and we studied at the same time embryonic fibroblasts obtained from Grb14−/− mice (MEF KO). Adipose differentiation, insulin signaling and adipokines's production and secretion have been observed in these two cell lines.
We demonstrate that adipose differentiation is not altered by Grb14 over expression. However, MEF KO lacking Grb14 show an increase both in cell proliferation and in adipose differentiation. Grb14 over expression leads to an inhibition of Akt and ERK1/2 insulin stimulated phosphorylation and its absence in the MEF KO enhances these same signaling events. Despite this effect on early insulin signaling, alteration of Grb14 expression level does not modify insulin induced glucose transport in both cell lines. Concerning the endocrine capacities of these adipocytes, we observe a 50% decrease in leptin secretion when Grb14 is overexpressed. On the other hand, adiponectin production and secretion are not altered by Grb14 expression level variations.
These experiments bring new knowledge about Grb14 function in adipose tissue, both on the metabolic and the endocrine aspects. Furthermore, they suggest that beyond it is role as an inhibitor of insulin signaling, Grb14 seems to act on other major cellular functions such as cell proliferation and adipose differentiation.

P554

25-Hydroxyvitamin D deficiency in premenopausal morbidly obese women

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Background
Obesity has been associated with low levels of 25-hydroxyvitamin D (25(OH)D). This could be explained by a low dietary intake, reduced sun exposure or less biodisponibility because of its arrest in the fat mass. The main objective is to study the prevalence of 25(OH)D deficiency in premenopausal obese women.
Methods
Aleatory sample of 100 morbidly obese patients. Selection criteria were: women, premenopausal, Caucasian. Patient with diabetes mellitus (pathologic oral glucose tolerance test or previous history), renal or hepatic failure and those treated with biphosphonates, calcium or vitamin D were excluded. 35 severely obese women (mean age: 36.6±11 year, mean BMI: 49.4±6.6 kg/m^2) were enrolled. Anthropometric data were measured and the body composition was estimated by bioelectrical impedance. 25(OH)D level >30 ng/ml was considered normal, < 20 ng/ml as deficiency and between 20 and 30 ng/ml as a relative deficiency. Pearson's correlation coefficient was used to analyse the relationships between quantitative variables.
Results
Mean waist circumference was 131.5±12.8 cm. Mean percentage fat mass was 50.9±3.8%. Mean 25(OH)D level was 20.5±2 ng/ml.
Six of the studied women (17.14%) had normal 25(OH)D levels, 8 (22.86%) and 21 (60%) of them had relative deficiency and deficiency respectively.
25(OH)D levels presented a negative correlation with weight (r: −0.365; P: 0.04), BMI (r: −0.369; P: 0.038), waist circumference (r: −0.42; P: 0.021) and the fat mass percentage (r: −0.382; P: 0.031).
Conclusions
1. The prevalence of 25(OH)D deficiency in premenopausal morbidly obese women is high (60%).
2. The negative correlation of 25(OH)D levels with BMI, waist circumference and fat percentage suggests a possible implication of excess body fat mass in the apparition of 25(OH)D deficiency.

P555

Association study on three single nucleotide polymorphisms upstream and in the GIPR in obese and lean children from Berlin

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Introduction
In the past 20 years, obesity has become a major health problem occurring as well in adults as in children. Beside environmental influences on body weight, the genetic background of a person plays an important role in body weight control. After the description of monogenetic mutations linked to obesity most current studies investigate polygenetic forms of obesity.
Recent studies showed an association between two SNPs, located in the promoter region and in the first intron of glucose dependent insulinotropic peptide receptor gene (GIPR), and obesity in several cohorts (Broenner *et al. in prep.*). To further investigate the association of *GIPR* with body mass related phenotypes, we genotyped these two SNPs and the additional rs1800437 located in exon 12 of *GIPR* in a case–control sample of obese and lean children.
Methods
The study sample included 600 obese children and adolescents (age 2.5–18 years; BMI >95th percentile) who are patients of the obesity out patient's clinic of the Otto-Heubner-Centrum in Berlin (257 male) and 1600 lean children in the same age range from Berlin (72 male; BMI-SDS ±0.1). Genotyping was either performed using the SNaPshot protocol (Applied Biosystems) or using the iPLEX and MALDI TOF technique (Sequenom).
Results/conclusion
The data are currently under evaluation. So far we observe a trend towards association in the male subset only. There seems to be is a shift towards heterozygote forms for all three SNPs in obese boys. We detected no effect either for the complete or the female subset. The data are under evaluation in regard to sex, age and the national background of the children to avoid segregation bias.

P556

Screening for mutations in the glucose-dependent insulinotrophic polypeptide receptor gene (GIPR) in obese patients with disturbed glucose tolerance

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Objective
The increasing incidence of obesity is a major health problem world-wide. So far, mutations were identified in genes encoding major contributors in energy homeostasis. With the exception of mutations in the *melanocortin-4-receptor gene* (*MC4R*) obesity-causing mutations are very rare. To date great efforts were undertaken to identify gene variants that contribute to polygenic obesity. The GIPR belongs to the large superfamily of G-protein coupled receptors. It is mainly expressed in pancreatic beta cells and is involved in glucose-mediated insulin secretion. Activation with GIP leads to signalling via the

Gs/adenylylcyclase pathway. In mice target disruption of GIPR resulted only in modest alterations in glucose homeostasis. However, overexpression of a dominant-negative *GIPR* mutation in mice severely interfere with pancreatic beta cell development and resulted in a diabetic phenotype. Recent studies provide evidence that decreased insulin sensitivity and the risk for cardiovascular disease is enhanced when GIPR expression is reduced.
Patients/methods
We investigated 150 obese children including patients with disturbed glucose tolerance from our out-patients clinic for sequence variation in the *GIPR*. The 14 exons of the coding region including the exon/intron boundaries were PCR-amplified and directly sequenced.
Results/conclusion
We detected 5 known and two novel single nucleotide polymorphisms (SNP). Additionally, we identified 32 heterozygous and 3 homozygous carrier of the non-synonymous amino acid exchange Glu354Gln in exon 12 (transmembrane domain 6). Functional studies are ongoing to understand the functional role of identified sequence variations in the *GIPR* for obesity and glucose homeostasis.

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Analysis of the central glucocorticoid feedback in patients with obesity, diabetes, and depression

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Obesity, diabetes, and depression are associated with distinct neuroendocrine changes influencing the regulation of the hypothalamus pituitary adrenal (HPA) system. Up to now it was not possible to examine the central glucocorticoid feedback in humans to verify results of animal experiments in relating disease models. With the help of a recently developed computational approach we were enabled to address this question to patients with obesity, diabetes, or depression. In order to identify the glucocorticoid feedback and to analyse the kinetics of corticotropin and cortisol we established a system of differential equations. A corticotropin releasing hormone test was applied to generate the data for the analyses.
We found that subjects with obesity, depression, type 1 diabetes, or insulin treated type 2 diabetes have specific changes of central glucocorticoid feedback parameters. These changes of feedback parameters indicate an altered setpoint regulation of the HPA system in humans with psychiatric and metabolic diseases.

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Evaluation of metabolic syndrome in patients with diabetes mellitus type 2 using different criteria: National Cholesterol Education Program's Adult Treatment Panel III (ATPIII) and International Diabetes Federation (IDF)

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The aim of the study was to compare ATPIII and IDF criteria for metabolic syndrome in diabetes mellitus type 2 subjects.
Two hundred and seventy-seven participants were included, 177 women 60.43 ± 8.06 years and 100 men aged 56.35 ± 9.58 years. Group A: metabolic syndrome by ATPIII; group B metabolic syndrome by IDF.
Following laboratory and antropometric measurements were done: total cholesterol, triglycerides, LDL- and HDL cholesterol, fasting plasma glucose, body height, body weight, waist circumference, arterial blood pressure.
We found fewer subjects with metabolic syndrome in group A compared to group B (65% versus 68%). Group B had more women (78%) than group A (71%). In males, it was vice versa (group A 54% versus group B 51%).
Male subjects of group A had statistically significant ($P<0.05$) smaller waist circumference and mean daily glucose levels compared to male subjects in group B (101 ± 11.54 vs 104 ± 7.64 and 12.89 ± 4.36 vs 14.52 ± 3.94, respectively). Group A had more atherogenic lipid profile (TG 2.76 ± 1.79 in males and 2.59 ± 1.99 females, HDL 1.08 ± 0.13 in males and 1.22 ± 0.3 in females, LDL/HDL 3.71 ± 1.43 in males and 3.27 ± 0.89 in females) compared to group B (TG 2.64 ± 2.01 in males and 2.63 ± 1.96 in females, HDL 1.14 ± 0.33 in males and 1.24 ± 0.31 in females, LDL/HDL 3.42 ± 1.15 in males and 3.23 ± 0.92 in females), but without statistic significance. We did not find statistically significant difference in arterial blood pressure values between two groups ($P>0.05$).
The use of ATP III and IDF criteria did not show statistically significant difference in detecting metabolic syndrome in all diabetes mellitus type 2 participants ($\chi^2=0.659$; $P>0.05$). There was not any statistically significant difference between women and men ($\chi^2=2.16$; $P>0.05$ and $\chi^2=0.08$; $P>0.05$, respectively).

P559

Relation of high-sensitive C-reactive protein and metabolic risk factors in overweight and obese women

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Introduction
Elevated high-sensitive c-reactive protein (hs-CRP) in association with hyperinsulinemia, obesity, insulin resistance and high blood pressure are significant risk factors for cardiovascular diseases. The aim of this study is to evaluated the correlations between serum hs-CRP concentration and metabolic risk factors in overweight and obese women.
Methods
Overweight (BMI > 25 kg/m^2) and obese (BMI > 30 kg/m^2) 755 women were - enrolled into the study. The patients were divided into two groups according to serum hs-CRP concentrations. The mean serum hs-CRP concentration was 3.4 mg/dl. Group I ($n=371$) consist of serum hs-CRP ≤3.4 mg/dl and, group II ($n=384$) consist of serum hs-CRP ≥3.4 mg/dl. The groups were compared for metabolic risk markers regards of cardiovascular disease.
Results
The mean serum hs-CRP concentration was 3.4 mg/dl. Weight, body mass index (BMI) waist circumference, sagital waist, body fat, blood pressure, insulin, HOMA, WBC and uric acid were significantly high in Group II compared to Group I ($P<0.05$) there was no difference for glucose, cholesterol, triglyceride, HDL, LDL, kreatinin, ferritin between the groups.
Conclusion
Here in this study, we defined the correlation between increment of serum hs-CRP concentrations and metabolic risk markers regards of cardiovascular risk. These data suggest that elevated plasma hs-CRP levels might be associated with CVD risk factors in overweight and obese women.

Table The comparison of different hs-CRP groups.

Parameter	Group 1 ($n=371$)	Group 2 ($n=384$)	*t* value
Age (years)	41.50 ± 11.71	42.11 ± 11.82	NS
Weight (kg)	86.71 ± 14.37	98.09 ± 18.07	<0.001
BMI (kg/m^2)	34.45 ± 5.67	39.66 ± 7.19	<0.001
Uric acid (mg/dl)	4.22 ± 1.06	4.61 ± 1.08	<0.001
Systolic BP (mmHg)	127.43 ± 22.34	132.53 ± 21.94	0.002
Diastolic BP (mmHg)	83.44 ± 35.09	85.97 ± 35.11	0.043
Glucose (mg/dl)	95.85 ± 29.64	97.27 ± 24.47	NS
Insulin (uIU/ml)	12.19 ± 10.30	15.21 ± 12.38	0.001
HOMA (log)	3.18 ± 4.92	3.74 ± 3.39	<0.001

P560

Relationship of visceral adiposity with plasma adiponectin concentration: effect of weight loss

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Objective
To investigate the associations between adiponectin levels and adiposity and body fat distribution indices assessed by waist to hip ratio in Iranian women and to determine if the association differ as a result of ethnicity.
Methods
A cross-sectional study of 76 Iranian women free from metabolic disease was performed. Body mass index (BMI), waist circumferences, waist to hip ratio (WHR), body composition, insulin sensitivity, lipid profiles and plasma concentration of adiponectin were measured. Adiponectin changes after a weight loss diet in a subgroup of 42 obese subjects was also assessed.
Results
WHR (waist to hip ratio) was the only variable independently associated to adiponectin (Beta $=0.25$, $P<0.05$). A mean increase of $8.2\pm24.2\%$ in adiponectin concentration was observed in response to the dietary restriction and weight loss ($P<0.05$).
Conclusions
Our findings provide evidence for association of serum adiponectin level with visceral fat, represented by waist to hip ratio index. Our results also indicate that moderate weight loss result in significant improvements in adiponectin concentration. This provide another biological explanation for the beneficial effect of body weight loss on reducing cardiovascular and diabetes risks in obese patients.

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The interrelationship between leptin, visfatin and CRP levels and insulin sensitivity assessed by euglycaemic clamp in obese women

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Visfatin is a newly discovered adipokine found in abundance in visceral fat. Leptin is well known marker of fat mass in the body but also one aspect of leptin is its action as a proinflammatory cytokine. Recent studies, both *in vitro* and *in vivo*, indicated that visfatin is also one of the inflammatory cytokines, although the relationship between visfatin and insulin resistance remains still unclear. The aim of our study was to assess the association between visfatin and leptin levels in circulation and those of C-reactive protein (CRP), as marker of systemic inflammation, and also to investigate their relationship with insulin sensitivity index in obese women. In that order, thirty obese women (BMI$=35.59\pm0.83$ kg/m^2, age$=35.53\pm1.59$ years) were included in the study. In each of the investigated subjects, following parameters were measured: Visfatin (EIA Phoenix, ng/ml), Leptin (Linco RIA, ng/ml), hs-CRP and M index (mg/kg per min) during euglycemic clamp studies as a marker of insulin sensitivity. Significant positive correlation was found between leptin levels and and CRP ($r=0.435$, $P<0.05$) while there were no correlation between leptin and M index ($r=-0.128$, $P>0.05$). There were no significant correlation between visfatin and CRP ($r=-0.029$, $P>0.05$), while visfatin significantly correlated with insulin sensitivity (M index) ($r=0.309$, $P=0.055$). There was significant negative correlation between CRP and M index ($r=-0.439$, $P=0.001$). In conclusion, according our results, visfatin levels are in significant positive correlation with insulin sensitivity index calculated during euglucaemic clamp, while there was no significant correlation between visfatin and CRP in obese women. Leptin seems to be a better marker of inflammatory state than visfatin in obese women.

P562

Obesity and adipocytokines

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Aim
Comparison of the parameters of obesity with the newly-defined adipocytokines in females applying to our clinic for obesity.
Method
Demographical data of 36 obese females were determined. Cases were classified as obese and morbid obese according to BMI values. Biochemical parameters were measured for all the cases. Thicknesses of subcutaneous and visceral fatty tissues were measured on ultrasonography. Statistical significances were evaluated by applying Pearson correlation and Student *t*-test.
Findings
According to the correlation analysis of the entire group, it was seen that plasma visfatin and apelin measurements had no correlation with parameters other than the correlation between them ($r=0.42$, $P<0.05$), and plasma leptin levels correlated positively with BMI, subcutaneous and visceral fatty tissue measurements, and h-CRP. In the correlation analysis of the obese group, it was found that there was correlation between apelin and visfatin; and in the morbid obese group, there was correlation between apelin and systolic blood pressure and fasting–postprandial glucose and visceral fatty tissue and h-CRP. When obese and morbid obese groups were compared, it was found that there were no statistically significant differences in plasma visfatin and apelin levels, although there were significant differences between BMI, waist circumference, systolic blood pressure, postprandial glucose, h-CRP, leptin, and thicknesses of subcutaneous and visceral fatty tissues.
Conclusion
In our study, increasing of plasma leptin levels with the increasing degree of obesity, and positive correlations with body mass and fatty tissue measurements are consistent with the literature. No correlation was found in our study between the levels of plasma visfatin and apelin levels and BMI, body fat amount, and thicknesses of subcutaneous and visceral fatty tissues, and no significant changes were seen between morbid obese and obese groups regarding the same. We believe that further studies are required to enlighten this issue.

P563

ERK1/2 MAPKs and Wnt signaling pathways are independently involved in adipocyte-mediated aldosterone secretion

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One important risk factor for the development of arterial hypertension is abdominal obesity. Aldosterone, secreted by adrenocortical cells, promotes sodium and water retention and by that regulates blood pressure homeostasis. Increased serum aldosterone levels have been linked to the development of obesity hypertension. Therefore, identifying the link between obesity and increased aldosterone secretion is of high importance for the management of obesity hypertension.
Isolated human adipocytes secrete factors that stimulate steroid secretion from human adrenocortical cells with a predominant effect on aldosterone secretion.
We could show that adipocyte-mediated aldosterone secretion and sensitization of human adrenocortical cells to angiotensin II (AngII) is mediated via ERK1/2-MAPKs-dependent upregulation of steroidogenic acute regulatory (StAR) protein activity. Inhibition of MAPKs by UO126 almost completely abolished adipocyte-induced steroidogenesis. Recent evidence also indicates the involvement of the Wnt-signalling pathway in fat cell-mediated aldosterone secretion.
We then evaluated possible interactions of the ERK1/2 MAPKs and the Wnt signalling pathways in adipocyte-induced adrenocortical aldosterone secretion. Exposure of human adrenocortical NCI H295R cells to Wnt-3a did not affect ERK 1/2-activation. Accordingly, fat cell-induced ERK1/2 phosphorylation was not inhibited by the Wnt-antagonist sFRP-1.
Therefore, ERK1/2 MAPKs pathway and the Wnt signalling pathway seem to be two independent mechanisms in adipocyte-mediated aldosterone secretion. These mechanisms might be important in the development of obesity hypertension.

P564

Leptin decreases and glucose deprivation increases the generation of the soluble leptin receptor (sOb-R) in a cell model

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Objective
The soluble leptin receptor (sOb-R) is generated by ectodomain shedding of the membrane-associated Ob-R and may modulate leptin action in metabolic dysregulations. The objective of our study was to investigate whether or not sOb-R generation may be induced by indicators of energy availability or metabolic regulators in a cell model.
Material and methods
The four human Ob-R isoforms were cloned, characterized by binding experiments with 125-iodinated leptin and transfected into HEK 293 cells. Cells were stimulated with levels of leptin, glucose, phorbol 12-myristate 13-acetate (PMA), insulin, human growth hormone, dexamethasone, troglitazone and AICAR. Ob-R gene expression was controlled by an isoform specific TaqMan assay. sOb-R levels in cell supernatant were measured by an in-house immunofunctional assay.
Results
Leptin incubation of our Ob-R transfected ($P<0.01$) and non-transfected cells ($P<0.05$) lead to a concentration-dependent significant decrease of sOb-R. Total glucose deprivation and PMA stimulation were associated with increased sOb-R results, whereas low levels of glucose restored the normal shedding function of the cells. No significant effect on sOb-R was observed after incubation with the remaining substances.
Conclusions
Presumed that sOb-R levels in supernatant of cells reflect the density of the Ob-R membrane receptor, the leptin-dependent decrease of sOb-R suggests receptor down-regulation or leptin resistance. This suggestion is in-line with *in vitro* data of obese subjects. In contrast, the increase of sOb-R by glucose deprivation may support the hypothesis that Ob-R shedding is associated with reduced energy availability of the cell. The consequence of both effects for the net action of leptin has to be determined in further experiments. Additionally, the increase of sOb-R concentration after PMA stimulation underlines that the shedding process is PKC dependent.

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The impact of middle term feeding with organic, low input and conventional diets on body composition and plasma leptin concentration in male rats

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The aim of the study was to analyze the influence of feed from different production systems on food intake, body chemical composition and plasma leptin concentrations in rats. It is expected, that organically produced feed influences positively rat organism, in that hormone balance. Leptin regulates energy intake and expenditure, as well as basic functions of many tissues.
The experiment was conducted in 104 Wistar male rats divided into 16 dietary groups (organic, conventional and two low input, all in four replications) and one control group (Labofeed H) consuming feed *ad libitum* for three months. Plasma leptin levels by RIA, body composition by standard chemical methods and total food intake and body weight gain were determined. Macronutrient and bioactive compounds intakes were also calculated. All procedures were approved by the Local Animal Care and Use Committee in Warsaw.
According to multifactorial statistical analyses, plasma leptin concentrations are observed to depend significantly on fertilization regime ($P<0.02$), while crop protection system does not influence significantly this parameter. At the same time, there is strong interaction between crop protection and fertilization regimes ($P<0.001$). Lowest plasma concentrations of leptin are observed in rats fed one of low input diets (feed from production system based on conventional fertilizers and organic protection) and standard diet (Labofeed H). Plasma leptin levels in other analyzed groups are statistically higher, however there are no significant differences between these groups. Moreover, the results show positive correlation between plasma leptin and β-carotene intake ($r=0.32$, $P\leq0.002$) and negative correlation between plasma leptin and dietary fiber intake ($r=-0.28$, $P\leq0.01$). Presented results do not confirm unquestionably earlier hypothesis. However, analyzed parameter is only one of many parameters which can be taken into consideration to evaluate the influence of organically produced food on the organism.

P566

Prospective, Placebo controlled, randomized treatment of 67 obese children/adolescents with metformin

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Background
It becomes increasingly evident that features of the metabolic syndrome are already present in young obese patients. Especially an impaired glucose metabolism with insulin resistance is an alarming sign of existing comorbidity of childhood obesity. Metformin has been argued as one pharmacological option to improve impaired glucose metabolism at least in obese adults. So far three prospective randomized studies were performed in childhood cohorts with <30 patients each (Kay 2001, Freemark 2001, Srinivasan 2006), suggesting a beneficial effect of metformin, however with low significances. To further gain evidence for the actual treatment with metformin we conducted a randomized, placebo controlled study in larger obesity childhood cohort.
Methods
We included obese non-diabetic children and adolescents (age range: 10–17 years.) with insulin resistance (HOMA$>$97. Perc.) and/or impaired glucose tolerance (total $n=243$). After an initial 6 month weight reduction program only those patients with unsuccessful weight reduction and persistent insulin resistance were recruited for the metformin treatment ($n=67$). The 67 patients were randomized and treated either with placebo or 2×500 mg metformin for 6 month. At baseline and after 6 month anthropometric (BMI-SDS, body composition, waist circumference) and metabolic parameters (oral glucose tolerance, HOMA, ISI, lipids) were measured.
Results
In 53% vs 36% (metformin vs placebo) the BMI and in 73% vs 54% (metformin versus placebo) HOMA was reduced after 6 month. But statistically comparing the placebo and treatment group did not revealed significant changes of all weight or glucose metabolic parameters (BMI-SDS, body composition, HOMA, ISI, lipids). Drop outs rate was 9% ($n=6$; $n=1$ metformin, $n=5$ placebo).
Conclusion
The lack of significant difference of all tested parameters of glucose metabolism in the metformin treatment vs placebo group of this so far largest metformin treatment cohort of obese children does not support the use of metformin in childhood obesity to improve insulin resistance, at least in addition to multiprofessional obesity programs.

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Hyperandrogenism in pre- and post-menopausal obese women

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Introduction
Obesity has been associated with increased androgenicity in women, however there are conflicting data on this subject. Objectives: To determine whether any androgen is independently related to body mass index (BMI), waist-to-hip ratio (WHR) or age. To access if there is an association between elevated plasma androgen levels and the presence of either morbid obesity or metabolic syndrome (MS).
Design and Methods
A total of 148 obese women were evaluated in their first obesity medical appointment: 105 premenopausal and 43 postmenopausal. Anthropometric variables, plasma androgen (total testosterone; T, free testosterone; FT, dehydroepiandrosterone sulfate; DHEA-S, androstenedione) and sex hormone

binding globulin (SHBG) concentrations were measured. The correlation coefficient and Fisher's exact test were used, respectively, to determine the strength of the linear relationship and to evaluate the non-random association among variables.
Results
The patients had mean age of 41.8±11.7 years and mean BMI of 40.5±7.5 kg/m^2; 60.1% of the women had morbid obesity. In premenopausal women, BMI was negatively correlated with SHBG ($r=-0.21$; $P<0.05$) and positively correlated with FT ($r=0.27$; $P<0.05$) and androstenedione ($r=0.25$; $P<0.05$). Age was negatively correlated with FT ($r=-0.26$; $P<0.05$), T ($r=-0.26$; $P<0.05$), DHEA-S ($r=-0.35$; $P<0.05$), androstenedione ($r=-0.33$; $P<0.05$) and T/SHBG ratio ($r=-0.34$; $P<0.05$). There was a positive correlation between WHR and DHEA-S ($r=0.2$; $P<0.05$). On the other hand, in postmenopausal women, there was only a negative correlation between BMI and SHBG ($r=-0.46$; $P<0.05$). There wasn't an association between elevated serum androgen levels and the presence of morbid obesity or metabolic syndrome (MS).
Conclusions
In this study it was found a correlation between BMI and age with some of the studied androgens, only in premenopausal women.

P568

Relationship between obesity, IL-17 and IL-23 and insulin resistance

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In most obese patients, obesity is associated with a low-grade inflammation of white adipose tissue resulting from chronic activation of the innate immune system and which can subsequently lead to insulin resistance, impaired glucose tolerance and even diabetes. It is still not clear role of acquired immune response in obesity and related comorbidites such as asthma, cancer or autoimmune diseases. The aim of our study was to compare level s of IL-17 and IL23 in sever obese women with nonobese healthy women as well as to investigate relationship between these interleukins with insulin sensitivity in same persons. In that order, thirty obese women (BMI=35.59±0.83 kg/m^2, age=35.53±1.59 yrs) with normal fasting glucose and 15 healthy control women (BMI=20.43±0.66 kg/m^2, age=27.87±0.77 years) were included in the study. In each of the investigated subjects, following parameters were measured: IL-17, IL-23, fasting glucose and fasting insulin, HOMA-IR as marker of insulin sensitivity. There was significant difference in circulating levels of IL-17 between obese and nonobese women (16.16±1.13 vs 9.42±1.16, $P<0.05$) as well as in IL-23 (9.35±1.87 vs 2.10±1.33, $P<0.05$). HOMA-IR was lower in control women but difference was not statistically significant (4.05±0.44 vs 2.95±0.48, $P>0.05$).There was significant correlation between BMI and IL-17 ($r=0.483$, $P<0.05$) as well as BMI and HOMA-IR ($r=0.415$, $P<0.05$). In conclusion, it can be considered that obesity corresponds to a sub-clinical inflammatory condition that promotes the production of pro-inflammatory factors primary involved in acquired immune response but further investigations are necessary to elucidate these interrelationship.

P569

NMR study of ghrelin and desacyl-ghrelin with the growth hormone releasing receptor type 1a using living cells

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Ghrelin, the endogenous ligand for the GH secretagogue receptor type 1a (GHS-R1a), is a peptide with a post-translational octanoyl modification on Ser-3. Previous studies of ghrelin derivatives showed that the pendant group at Ser-3 plays an essential role in the bioactivity. The acylated peptide specifically releases growth hormone (GH) both in vivo and in vitro, while the desoctanoyl form of the hormone is at least 100-fold less potent than the parent peptide.
Binding events of ligands to receptors are the key for understanding the biological processes. Gaining insight into protein-protein and protein-ligand interactions in solution has recently become possible on an atomic level by new NMR spectroscopic techniques.
In this work, the mapping of the interaction of ghrelin with its receptor by NMR techniques in living cells is presented. The evaluation between spectra with stably transfected and wild type cells of the same cell line, allowed a mapping of the interactions of ghrelin and desacyl-ghrelin with the target GHS-R1a receptor. Ghrelin was found to have a higher number of residues affected by chemical shift perturbation (CSP) or slow conformational exchange (SCE) effects by interaction with the receptor, and the *n*-octanoic group was seen to be clearly necessary for the interaction with the receptor, supporting the conclusion that the NMR data in living cells report accurately the functional interaction of these peptides. The large number of SCE effects detected for ghrelin: Ser3, Phe4, Leu5, Val12, Gln13/Gln14, Lys16/Lys19, Glu17 and Lys24, suggest that the binding to its receptor is accompanied of a large conformational change respect to the random coil structure described in free solution.

P570

C-reactive protein and insulin sensitivity in severe obese women with and without metabolic syndrome

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It was postulated that different therapeutic approaches could influence the markers of inflammation in obesity but it is unknown whether overweight women with and without metabolic syndrome have the same degrees of inflammatory state. The aim of our study was to compare level of hs-CRP in obese women without other components of metabolic syndrome with hs-CRP in women with metabolic syndrome (according IDF criteria). Overweight body mass index-matched women without (OW)) ($n=16$, (body mass index, 35.56±0.09 kg/m^2) and with (May ($n=13$) metabolic syndrome (body mass index, 6.76±1.57 kg/m^2) were included in the study. C-reactive protein (CRP) between groups was not significantly different (4.39±0.711 mg/liter for OW vs 6.02±1.55 mg/l for MSy, $P>0.05$). There was a significant difference in triglyceride levels (1.12±0.14 for OW vs 2.86±0.46 mmol/l for MSy, $P<0.05$, HDL-cholesterol (1.35±0.06 for OW vs 1.13±0.05mmol/l for MSy, $P<0.05$), waist circumference (102.6±2.82 cm for OW vs 112.31±3.03 cm for MSy, $P<0.05$), but not in HOMA-IR index (3.86±0.40 for OW vs 4.46±0.96 for Msy, $P>0.05$). There was no significant correlation between CRP and BMI neither between CRP and HOMA-IR. Our results couldn't confirm higher degree of insulin resistance between severe obese women with and without other component of metabolic syndrome as well as difference in CRP levels.

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Inflammatory parameters in obesity: lack of influence of weight loss

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Introduction
Obesity has been associated with a low-level activation of the acute-phase response. The aim of the present study was to compare inflammatory parameters in patients with obesity and a control group, and to evaluate the effect of weight loss on these parameters.
Methods
Sixty-seven severe or morbid obese patients (51 women, 16 men), aged 34±11 years, were compared with 67 normal-weight controls (45 women, 22 men), aged 32±10 years. After initial evaluation, the patients received treatment for 4 weeks with a very low-calorie diet (VLCD), followed by a low calorie diet (1200–1500 kcal/day) for 2 months. Exclusion criteria were organic, infectious or inflammatory disease, ischaemic heart disease or stroke, diabetes mellitus, hyperlipidaemia and hypertension. An anthropometric and analytical evaluation was performed before and after the diet, measuring fibrinogen, CRP, IL-6 and TNF-α. Student *t*-test was used to compare the differences between groups. Pearson correlation coefficients were calculated to describe the association between variables.

Results
Obese patients showed higher levels of CRP, IL-6, TNF-α, leukocyte and neutrophil count than controls. After adjusting for BMI, differences in CRP remained statistically significant. In obese patients, inflammatory parameters (leukocyte count, neutrophil count, and IL-6) were significantly correlated with anthropometric parameters (weight, BMI and waist). None of these correlations was observed in the control group. Sixty-two patients completed 3 months of diet with a mean percentage weight loss of 8.6%. No change was observed in any inflammatory parameter after weight loss.
Conclusions
Obesity is associated to a chronic inflammatory state, probably due to proinflammatory cytokines secretion. Moderate weight loss does not ameliorate this proinflammatory state.

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Activated protein C levels in obesity and weight loss influence

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Introduction
Several haemostatic disturbances have been described in obese patients. Nevertheless, the state of coagulation inhibitors has been scarcely studied in these patients. Activated protein C (APC) is the anticoagulant enzyme formed upon activation of the protein C (PC) by the thrombin-thrombomodulin complex on the surface of endothelial cells. Morbid obesity is associated to endothelial dysfunction, which could cause a reduction in APC generation.
Methods
Sixty-seven severe or morbid obese patients (51 women, 16 men), aged 34 $\pm$ 11 years, were compared with 67 normal-weight controls (45 women, 22 men), aged 32 $\pm$ 10 years. After initial evaluation, the patients received treatment for 4 weeks with a very low-calorie diet (VLCD), followed by a low calorie diet (1200–1500 kcal/day) for 2 months. Exclusion criteria were organic, infectious or inflammatory disease, ischaemic heart disease or stroke, diabetes mellitus, hyperlipidaemia and hypertension. An anthropometric and analytical evaluation was performed before and after the diet, measuring APC, PC and prothrombin fragment F1+2 as a marker of hypercoagulability. Student t-test was used to compare the differences between groups. Pearson correlation coefficients were calculated to describe the association between variables.
Results
Obese patients showed significantly higher levels of APC, PC and F1+2 ($P=0.001$, $P=0.015$ and $P=0.010$ respectively). After adjusting for BMI, differences in APC remained statistically significant ($P=0.047$). After three months of diet, a significant decrease in APC ($P=0.043$) and F1+2 ($P=0.025$) and a non significant decrease in PC ($P=0.067$) was observed.
Conclusions
APC levels are increased in obese patients, in part because of greater thrombin generation and higher PC levels. After weight loss, we have found a decrease in APC levels, due in part to a thrombin generation reduction.

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Determination of obesity and its relationship with hypertension among Semnanian adults, Iran

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Introduction
Obesity and its related diseases like high blood pressure are the main reason of coronary heart diseases that are the second main cause of mortality in I.R of IRAN. The significant rise of obesity in the last decade resulted in corresponding increase in the prevalence of hypertension.
Objective
To detect obesity and hypertension, and the relationship between them among Semnanian people, Iran, 2007
Methods
In this cross sectional survey we studied 388 (169 male and 219 female) 40–65 year-old on the basis of a stratified random sampling. Weight, height and systolic and diastolic blood pressure (BP) were measured based on standard methods and BMI was calculated. Overweight and obesity were defined as $30>\text{BMI}\geq 25$ and BMI ≥ 30 respectively. High blood pressure was defined, as systolic BP $>$ 140 mmHg or diastolic BP $>$ 90 mmHg. Data analysis was conducted using SPSS soft ware version 11.2.
Results
Mean of BMI among males and females were 26.8, 30.6 kg/m^2 respectively with significant differences ($P=0.0001$).The rate of overweight and obesity in total population was 79.3% (CI 95%: 75.2–83.3%) and among males and females were 68.5% (CI 95%:61.3–75.6%) and 87.5% (CI 95%:83.1–91.9%) respectively and the difference was significant ($P=0.0001$). The rate of high blood pressure among obese, overweight and others were 26.1%, 20.5% and 8% respectively and the differences were statistically significant ($P=0.007$). Significant linear correlation were detected between systolic blood pressure with BMI, and with age($r=0.11$, $P=0.02$ and $r=0.28$, $P=0.0001$ respectively) and diastolic blood pressure with BMI, and with age ($r=0.20$, $P=0.0001$ and $r=0.16$, $P=0.001$ respectively). After adjusting the results for age, this significant correlation was detected again between BMI with systolic and diastolic blood pressure ($P=0.004$, $P=0.0001$ respectively).
Conclusion
The prevalence of overweight, obesity and high blood pressure are high in this community, especially among women and urgent attention to suitable interventional program is a priority in health sector.

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Development of metabolic syndrome is associated with impaired quality of life: longitudinal data from the North West Adelaide Health Study

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Background and aims
Longitudinal data from the North West Adelaide Health Study (NWAHS) were used to examine the effect of development of metabolic syndrome between Stage 1 (2000–2003) and Stage 2 (2004–2006) on quality of life.
Material and methods
The NWAHS is a random, representative sample of people aged 18 years and over living in the north west region of Adelaide. Participants were recruited via telephone interviews to attend the clinic. Of the original cohort ($n=4060$), 94.3% were contacted at Stage 2, with metabolic syndrome status at follow-up obtained for 79.0% ($n=3206$) of original participants. Metabolic syndrome using IDF criteria was defined as waist circumference ≥ 94 for men or ≥ 80 cm for women plus two of: triglycerides >1.7 mmol/l; HDL cholesterol <0.9mmol/l for men or <1.1 mmol/l for women; blood pressure $>=130/85$; FPG$>=5.6$ or diagnosed diabetes. Quality of life was measured using the eight subscales of the Short Form 36 (SF-36).
Results
The prevalence of metabolic syndrome at Stage 1 was 20.1% (18.9–21.4). The three-year cumulative incidence of metabolic syndrome among those without metabolic syndrome at Stage 1 was 7.9%. Participants who had metabolic syndrome at Stage 1 and Stage 2 were significantly impaired on all SF-36 subscales except Role Emotional and Mental Health, compared to those who did not have metabolic syndrome. The change in quality of life over time was significantly different on the vitality subscale for those who developed metabolic syndrome when compared to those who did not.
Conclusions
Development of metabolic syndrome is associated with impaired quality of life. Management of factors contributing to metabolic syndrome should consider quality of life.

P575

Novel potent and selective non-peptide ligands of ghrelin receptor: characterization of endocrine and extraendocrine actions

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Several synthetic peptide and non-peptide ligands of the GHS-R1a have been described that release GH and stimulate food intake. Starting from a triazole scaffold, we have designed and prepared novel small molecules with high binding affinity to the GHS-R1a and we have investigated their effects *in vitro* on free intracellular calcium levels and in vivo on food intake and GH secretion in the rat. In CHO cells transfected with the GHS-R1a, hexarelin stimulated calcium levels in a concentration-dependent fashion. Hexarelin is a well characterized hexapeptide that was used as positive control. The compounds JMV2810, JMV3012 and JMV3021 given alone stimulated intracellular calcium levels, but in combination with hexarelin significantly inhibited its stimulatory effects. The GH stimulating activity of the compounds was studied in 10-day old rats, a model previously validated in our lab. Hexarelin, JMV2951, JMV3012 and JMV3021 significantly stimulated GH secretion. Given in association with hexarelin, JMV2951 blunted its GH-releasing effects, whereas JMV2810, JMV3012 and JMV3021 did not modify hexarelin activity. In young-adult rats, hexarelin effectively stimulated feeding behaviour. JMV2951 also stimulated food intake, whereas JMV2810, JMV3012 and JMV3021 had no effects. Interestingly, JMV2951 given in combination with hexarelin did not modify the stimulation of food intake induced by the latter, whereas JMV2810, JMV3012 and JMV3021 significantly inhibited hexarelin effects on feeding behaviour. In conclusion our results show that novel triazole agonists of the GHS-R1a might be endowed with effects on GH secretion divorced from those on feeding behaviour, further supporting the hypothesis that several receptors or signalling pathways might be involved. JMV2951, a potent GHS-R1a agonist stimulating both food intake and GH secretion, has been selected for further characterization of its effects in a rat cisplatin-induced cachexia model.

P576

Hypoadiponectinemia but not activation of immune markers is associated with impaired glucose metabolism in morbidly obese patients

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Objectives
Obesity is the major risk factor for the development of impaired glucose tolerance (IGT) and type 2 diabetes mellitus (T2DM). In addition, increased circulating levels of cytokines and chemokines and decreased adiponectin levels are associated with IGT and T2DM. However, a large part of morbidly obese patient remain normoglycemic. Therefore, we investigated if this protection can be attributed to a lower grade of inflammation or higher adiponectin levels.
Methods
Glucose tolerance of morbidly obese patients ($n=2754$, body mass index ≥ 40 kg/m^2) was assessed by oral glucose tolerance tests. In a case-control design we compared levels of eight immune mediators and adiponectin from patients with IGT/T2DM ($n=52$) and normal glucose tolerance (NGT; $n=59$). Gene expression in peripheral blood was determined by quantitative RT-PCR, and serum concentrations of immune mediators and adiponectin were measured by ELISA and bead-based multiplex technology.
Results
About 54% of the patients in our morbidly obese cohort were normoglycaemic, while 14% were diagnosed with IGT and 32% with T2DM. There was no statistically significant difference in mRNA expression or serum levels of pro-inflammatory markers. Interestingly, we could demonstrate an association of NGT with higher adiponectin levels ($P=0.039$). Adiponectin levels were negatively correlated with interleukin (IL)-6 and macrophage chemoattractant protein (MCP)-1, but independent the other immune mediators.
Conclusions
Lower adiponectin levels were associated with IGT/T2DM, but there was no further increase in inflammatory markers with IGT/T2DM in morbid obesity. This suggests that in addition to chronic, low-grade inflammation, adiponectin is an important factor in the development of, or protection against, T2DM in obesity.

P577

Genetic determination of leptin, insulin and adiponectin levels in the Berlin Fat Mouse Inbred 860 line

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Understanding the complexity of polygenic obesity includes the aspect of the hormonal adaptation during the process of selection for high body weight. Therefore we focussed on the analysis of hormones regulating energy homeostasis, growth and insulin sensitivity in the Berlin Fat Mouse Inbred 860 (BFMI860) line which we use as a model for polygenic obesity. The mouse line had been selected for low protein and high fat content before it was inbred. Under a standard maintenance diet the endocrine profile of BFMI860 males is typical for the obesity status with highly increased leptin and insulin and reduced adiponectin levels compared to C57BL/6 (B6) males. In response to a high fat diet, BFMI860 males showed marked hyperleptinemia and hyperinsulinemia with high variability in glucose levels. Their adiponectin levels continued to decline under high fat diet.
To identify the genetic reasons underlying the BFMI860 phenotype a crossbred population of BFMI860$\times$B6 was generated. All F2 animals were fed a high fat diet and analysed regarding the endocrine profile. Mapping quantitative trait loci by linkage analysis has identified a chromosomal region that contributes to high body weight and fat mass, but not lean mass. Homozygous BFMI860 animals had higher leptin and insulin serum levels corresponding to increased body and fat mass. Interestingly, males of the BFMI860 phenotype had reduced adiponectin levels, as expected, while females did not show a reduction in adiponectin levels. The BFMI860 males had also higher average levels of insulin and glucose. In contrast, homozygous BFMI860 females seemed to be more insulin-sensitive and glucose-tolerant likely due to their higher adiponectin levels. We concluded that homozygous BFMI860 males could have a higher risk to become insulin-resistant. Further linkage analysis between the hormone concentrations and segregation of genotypes is in process to find the genetic factors influencing these hormones.

Paediatric endocrinology

P578

The prevalence of endocrine complications in patients with thalassemia major

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Background
Frequent blood transfusions and iron overload lead to many complications in patients with -thalassemia major. Endocrine disorders are detected in these patients with a high frequency. Early diagnosis and treatment of these complications could result in improvement of quality of life.
Patients and methods
A total of 183 patients with thalassemia major aged between 10 and 22 years old were evaluated for endocrinopathy. Blood samples were taken for determination of serum T4, TSH, FBS, Ca, Ph, cortisol, FSH, LH, estradiol and testosterone levels.Medical charts were used to assure the regularity of blood transfusion and serum ferritin levels. Data were analysed using chi-square, independent t-test, ANOVA and logestic regression.
Results
In 62.29% of patients at least one form of endocrinopathy (other than delayed puberty) was detected. Other endocrinopathies in order of frequency were: growth failure (36.06%), hypoparathyroidism (16.93%), adrenal insufficiency (12.50%), diabetes mellitus (11.47%) and hypothyroidism (8.74%).
There was no significant relation between serum ferritin levels and endocrine disorders; except delayed puberty in males ($P=0.030$) and hypoparathyroidism in both genders ($P=0.040$). Although there was no relation between endocrino-patheies and the age at which Desferal therapy was introduced, there was a significant relation between both growth failure and diabetes mellitus with gender (P value $=0.007$ and 0.013, respectively).
Conclusion
This study showed that patients with thalassemia major (are) affected by multiple endocrine disorders. Only some of these endocrinopathies have association with increased levels of serum ferritin. Therefore, patients with thalassemia major need to be evaluated for endocrine disorders periodically, even when serum ferritin levels are normal.

P579

The role of thiocyanate in the etiology of residual goiter in Semirom, an iodine replete area

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Background
Despite long standing iodine supplementation in Iran the prevalence of goiter remains high in some areas. This may suggest that factors other than iodine deficiency may play a role. In the present we investigated the possible role of thiocyanate (SCN) in the etiology of goiter in Semirom, Iran.
Methods
One thousand and eight hundred and twenty-eight schoolchildren (7–13 year-old) were selected by multi stage random sampling. Thyroid size was estimated in each child by inspection and palpation. Urinary iodine concentration (UIC) and Urinary thiocyanate (USCN) were measured
Urinary SCN and iodine concentration was estimated by the method of Aldridge and digestion method
Results
Overall, 36.7% of 1828 students had goiter. The mean UIC was 19.3 ± 9.1 μg/dl. USCN level was estimated in 130 randomly selected schoolchildren (86 goiterous and 44 nongoiterous). The mean $\pm$ S.D. USCN level in goiterous and nongoiterous subjects was 0.77 ± 0.80 and 0.64 ± 0.40 respectively ($P = 0.67$). USCN level in goiterous and nongoiterous boys was 0.75 ± 092 mg/dl and 0.72 ± 0.45 mg/dl respectively ($P = 0.05$). USCN level in goiterous and nongoiterous girls was 0.75 ± 0.92 mg/dl and 0.72 ± 0.45 mg/dl respectively ($P = 0.33$).
Conclusion
In the studied population, thiocyanate overload may play a role in high persistence of goiter in boys. In girls it can not explain the still high prevalence of goiter. We suggest the role of other goiterogenic factors should be investigated in this region.

P580

Structural study on the effect of maternal diabetes on fetal endocrine pancreas

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The effect of maternal diabetes on fetal pancreatic islets was investigated before, however an extensive and detailed quantitative immunocytochemical investigation was not found. Therefore, this investigation was conducted to examine the effect of gestational diabetes on the morphology of fetal rat islets. Sections were stained with anti-insulin (B cells) antibodies and were used for structural study. The absolute number of stained B cells per islet of the diabetic group was not significantly different from that of the control group. The B cell volume density was significantly lower in the diabetic group, while islet volume density, islet diameter, islet volume and absolute islet cell number were significantly greater in diabetic group. The nuclear diameter and volume of B cell were not significantly different in the diabetic group. The results obtained from this study demonstrated that maternal diabetes induces fetal islet hypertrophy and B cell hyperplasia. They further showed that maternal diabetes results in a decrease of fetal islet insulin content.

P581

Successful treatment of isolated growth hormone deficiency type 1a with recombinant human growth hormone

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Isolated growth hormone deficiency (IGHD) type 1a is caused by defects of the GH-I gene resulting in severe short stature. Treatment with growth hormone may become ineffective due to significant production of growth hormone antibodies. However, this has only been reported in patients treated with pituitary derived growth hormone. We present five cases with IGHD type 1a successfully treated with recombinant human growth hormone (rhGH).
Five patients from four families (four females, one male) showed severe short stature beginning in the first year of life. All of them had undetectable levels of growth hormone and were subsequently found to be homozygous for a 6.7 kb deletion in the GH-1 gene. Two families were known to be consanguineous. Treatment with rhGH normalized growth in all subjects with a current duration of treatment of 1–10 years (mean 5.25 years). One patient has reached final height (initial height SDS – 7.92 at age 2 10/12 years; final height SDS 0.98).
Another patient (initial height SDS – 8.83 at 1 11/12 years; current height SDS 0.9 at 9 5/12 years) has two affected brothers who already had high titers of GH antibodies after having possibly been treated with pituitary derived GH. They remained unresponsive to rhGH even in excessive doses of up to 0.35 mg/kg.
In our experience treatment of IGHD type 1a with rhGH is not associated with a risk of GH antibody production leading to growth arrest. Prior to considering IGF-I as first-line treatment in IGHD type 1a further experimental and epidemiologic studies should evaluate the risk of antibody production on rhGH treatment.

P582

Polyendocrinopathy in children, adolescents and young adults with type 1 Diabetes: results from 23837 patients in the German/Austrian DPV-Wiss-database

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Few large-scale multicenter data on additional immune phenomena in patients with type-1 diabetes are available. The DPV initiative aggregates standardized anonymized patient records for quality control and epidemiologic research. This report includes data on 23 837 patients with type-1 diabetes, age <30 years and at least 1 antibody measurement, from 242 specialized centers from Germany and Austria (8012 patients <12 years, 12 866 12–18 years and 2959 patients 18–30 years). At least one B-cell-antibody (ICA, GAD, IA2, IAA at onset) was present in 10 133 patients. B-cell-AB-negative patients were significantly younger at diabetes onset (8.4 versus 9.1 years, $P<0.0001$). 2459 patients (10.3%) had positive thyroid antibodies (TAK, TPO) with female predominance (62%, $P<0.0001$) and association to older age and longer duration of diabetes (both $P<0.0001$), but no difference with age at onset. Thyroid-autoimmunity was not associated with positivity for any B-cell-AB, however the number of B-cell-AB + s detected was slightly higher in patients with thyroid autoimmunity (1.8 versus 1.6, $P<0.0001$). Antibodies suggestive of celiac disease (tTG, gliadin IgG/IgA or endomysium) were present in 3923 patients, with a significantly younger age at onset (7.5 versus 8.2 years, $P<0.0001$). Parietal cell antibodies were found in 244 patients, again associated with older age (15.9 versus 14.1 years, $P<0.001$). Adrenal antibodies were found in 71 patients, this group did not differ clinically from patients without adrenal antibodies. In 352 patients (61% female, mean age 14.6 years), at least 3 different autoimmune phenomena were present, and 4 organ systems were involved in 33 patients. In conclusion, Thyroid autoimmunity is the most prevalent additional immune phenomenon in type-1 diabetes, especially in adolescent and young adult women. Parietal or adrenal antibodies are rare. B-cell-autoimmunity is not a predictor for additional autoimmune phenomena in type-1 diabetes.

P583

Comparison of vitamin D status, sun-exposure and personalised UVB-radiation dosimetry of mildly pigmented, breastfed newborns during their first 6-8 weeks of life in Perth, Western Australia and Berlin, Germany

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The prevalence of vitamin D deficiency in at-risk groups is rising even in sunny countries. Aim of this study was to compare vitamin D status and effect of vitamin D supplementation of moderate risk (mildly pigmented, WHO skin type 2) breastfed newborns from sunny and temperate climate zones. Newborns in Perth (30°S, group 1:5 male, 6 female) did not receive any vitamin D supplementation, newborns in Berlin (52.5°N, 17 male, 23 female) were randomised into $n=20$ on 250 units (group 2) and $n=20$ on 500 units (group 3) of vitamin D3 per day.
We compared serum 25 OH vitamin D (25OHD), alkaline phosphatase, albumin; serum and urine calcium, phosphate and creatinine; sun exposure in hours/day and UVB exposure (minimal erythema dose, MED). Measures were obtained on day 5 and 6-8 weeks after delivery. UVB exposure was continuously quantified by spectral analysis using bio-weighted dosimeters. Surrounding factors and nutrition were assessed by questionnaires and analysis of meteorological data.
25OHD levels did not vary significantly between groups on day 5 (nmol/L $\pm$ S.E.M., groups1/2/3): $49.2\pm7.7/68.9\pm8.1/64.1\pm5.8$; 6–8 weeks later only newborns in Perth presented with subclinical vitamin D deficiency: 45.9 ± 14; group2:128.8 ± 10.6, group3:151.1 ± 18.3, $P<0.05$. Sun exposure time (h/day $\pm$ S.E.M.) was significantly lower in Perth: $0.5\pm0.08/2.14\pm0.13/1.7\pm0.3$, $P<0.05$. Dosimetry revealed similar UVB exposure (MED/day $\pm$ S.E.M.) for all groups: $0.03\pm0.02/0.04\pm0.02/0.03\pm0.02$. The remaining parameters were normal in all participants.
Newborns in Perth did not achieve sufficient 25OHD levels despite living in a sunny climate. To improve vitamin D photobiosynthesis their sun exposure can be optimised without increasing the risk of skin cancer; otherwise vitamin D supplementation is needed. In Berlin, Germany, 250 units of vitamin D3 per day resulted in sufficient vitamin D supply.

P584

Growth hormone deficient children born small-for-gestational-age need higher than replacement dose of hGH for successful treatment

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Growth hormone deficient (GHD) children born small-for-gestational-age (SGA) receive the same hGH dose as the children born with appropriate weight/length for gestational age (AGA). There are very few data on their lower growth response to the usual GH replacement dose. The aim of this multi-centre retrospective study is to analyse the hGH dose dependency of GHD children with SGA.
SGA was defined as a birth weight/length below – 2 SD for gestational age compared to the national standard. The data of hGH treated GHD (GH peak <7 ng/ml in two provocative tests) children and Turner syndrome (TS) with SGA. Some characteristics of the 41 prepubertal children (treated with hGH over 2 years) are the following: age: 8.37 ± 3.72 (2.71–15.87) years; sex: boy/girl 18/23; HSDS: -3.49 ± 1.0 (between – 1.7 and – 5.56); hGH dose: 0.69 ± 0.18 (0.4-1.05) IU/kg/w; ΔHSDS/2 years: 0.76 ± 0.74 (between -0.42 and $+2.48$).
The ΔHSDS was found 0.92 ± 0.77 in 13 children treated with a mean dose $\geq$0.8 IU/kg per w and 0.68 ± 0.73 in the other 28 children received $<$0.8 IU/kg per w. The same parameters proved to be 1.2 ± 0.6 and 0.73 ± 0.5 resp. in the younger (<6 years) age ($P<0.01$). This dose dependency was more significant in the children to be SGA for birth weight: growth response of 15 among 21 was <1.0 ΔHSDS/2 yrs treated with <0.8 IU/kg per w ($P<0.01$).
These results confirm the previous data of de Zegher *et al*. (1998), that the great majority of GHD children with SGA need higher than replacement dose of hGH for optimum growth response, probably because of some IGF-I resistance of these children.

P585

Autosomal dominant hypophosphatemic rickets (ADHR) due to a novel mutation in the FGF23 gene

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Two dominant inherited disorders of phosphate homeostasis, X-linked hypophosphatemia (XLH), and ADHR are known to be caused by inactivating mutations in the PHEX gene or activating mutations in the FGF23 gene (fibroblast growth factor 23), respectively. Both diseases show a similar phenotype with renal phosphate wasting and inappropriately normal or low 1,25-$(OH)_2$-Vitamin D_3 serum levels, leading to hypophosphatemic rickets and osteomalacia. The differential diagnosis can be achieved by molecular genetic analysis of the involved genes.
The index patient presented at the age of 2 years with growth retardation (-3.1 SDS; 4 cm <3. Percentile), genua vara, severe rickets, and osteomalacia, leading to bone pain and restricted mobility. Serum phosphate levels were low (0.8 mmol/l; normal range 1.1–2.0 mmol/l), tubular phosphate reabsorption (58%) and tubular phosphate transport maximum (0.8 mmol/l; normal range 1.2–2.6 mmol/l) were decreased. Calcium, 1,25-$(OH)_2$-Vitamin D_3 and PTH levels were normal. Based on these findings, hypophosphatemic rickets was diagnosed and the boy was treated with phosphate (550 mg/d) and 1,25-$(OH)_2$-Vitamin D_3 (0.4 µg/d). During treatment, radiological signs of rickets and osteomalacia decreased. The growth rate improved, although body height at the age of 7 years remains low (3. Percentile).
Mutations in the PHEX gene were excluded, but analysis of the FGF23 gene revealed a novel heterozygous missense mutation in exon 3, resulting in the substitution of the amino acid arginine at position 176 by tryptophane (CGG$>$TGG, R176W). The mutation R176W affects the cleavage site of FGF23 that is formed by the ^{176}RXXR^{179} motif, and is predicted to prevent proteolytic cleavage and inactivation of FGF23. In conclusion, in this patient genetic testing allowed for the reliable differentiation of ADHR and XLH.

P586

The role of desmopressin test in the diagnosis of young patients with Cushing's disease

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In the diagnosis of Cushing's disease (CD) desmopressin (dDAVP) stimulation test may be a convenient and more readily available alternative to CRH test; however the sensibility and specificity of ACTH response to dDAVP test has been reported in adult patients inferior to CRH test. In childhood patients with CD there are no data for the use of dDAVP test in this clinical setting. We studied 9 patients ranging in age from 11 to 19 years (7 females and 2 males) with a suspected diagnosis of CD. Preoperative endocrine assessment included CRH, dDAVP, overnight 8 mg dexamethasone tests. CRH and dDAVP tests (positive if ACTH and cortisol levels were respectively more than 50% and 20% of the baseline) resulted both positive in 8/9 cases but a more relevant mean increase has been found for ACTH levels after dDAVP stimulation. In all patients we observed a positive response (drop in cortisol levels more than 50% of baseline values) to the dexamethasone test. At MRI scans 6 patients presented a normal pituitary gland 3 a microadenoma. All the patients underwent transphenoidal neurosurgery that was successful in 7/9 (at hystology 6 adenomas and 1 normal pituitary tissue), the other 2 patients underwent pituitary radiotherapy. Conclusions: In children and adolescents with CD a same percentage (88%) of positive response to dDAVP and CRH stimulation tests was observed. Moreover an high percentage of negative MRI scans was found and in these cases the combined positive response to the CRH and dDAVP stimulation tests, and particularly the marked response to the latter, were useful tools in the diagnosis of CD

P587

Evaluation of Intergrated [^{18}F]FDOPA–PET/CT for identification of focal forms of Congenital Hyperinsulinism (CHI)

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Objective
CHI is the most frequent cause of severe hypoglycaemia in infants. Two distinct anatomical forms have been described which require different therapeutic strategies. We evaluated the predictive value and accuracy of integrated [^{18}F] FDOPA-PET/CT as a new tool in identification of focal lesions in an observational study.
Patients and methods
From 2005 to 2007, 73 infants and children from the UK (30) and Germany (42) with CHI were examined with [^{18}F]FDOPA-PET/CT for localization diagnostic. Since 2007 a high-resolution 64line CT-scan were used. A special angiographic enhancement protocol was developed and anatomical landmarks were identified to improve the localization within the organ and to guide the surgeon.
Results
In 69 of 72 patients a differentiation between focal and diffuse form was possible. Focal cases were treated surgically. The diagnosis was histological confirmed in 22 of 23 focal cases. In 9 cases the precise localization by the [^{18}F]FDOPA-PET/CT enabled minimal invasive laparoscopic surgery.
Subtotal pancreas resection was necessary in 3 diffuse cases while the others are manageable with medical therapy so far. We present the metabolic and neurologic outcome of the cases.
Conclusion
Integrated [^{18}F]FDOPA-PET/CT was able to discriminate between focal and diffuse forms in 95% of the cases and accurately localize the lesion in focal CHI. It is a valuable tool to support the determination of further therapy (medical or surgical) and finally to guide the surgeon in limited pancreatic resection.

P588

Epigenetic defects at GNAS DMRs in PHP-Ia patients lacking coding GNAS mutations

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Pseudohypoparathyroidism (PHP) is a disorder characterized by hypocalcemia and hyperphosphatemia due to end-organ resistance to the action of PTH. The two main subtypes of PHP, PHP type Ia and Ib are caused by heterozygous loss-of-function mutations in GNAS exons 1–13, which encode Gsα, and by methylation defects in the imprinted GNAS cluster, respectively. Individuals affected with PHP-Ia typically show clinical abnormalities referred to as Albright hereditary osteodystrophy (AHO) together with resistance toward several additional hormones, such as TSH, gonadotropins and GHRH. PHP-Ib differs from PHP-Ia in that affected patients do not have the AHO phenotype and hormone resistance appears to be limited to the actions of PTH and, occasionally, TSH. About 30% of patients with PHP-Ia features lack GNAS coding mutations. We investigated the presence of typical PHP-Ib epigenetic defects at GNAS differentially-methylated regions (DMRs) in 10 PHP-Ia cases lacking GNAS mutations. We found loss of methylation at A/B DMR in 6 cases and defects at other GNAS DMRs in 3 of them. In one patient we found the presence of STX16 gene 3.3 kb deletion, that characterizes familial PHP-Ib cases.
In conclusion, we confirm that GNAS methylation defects can explain about 50% of PHP-Ia cases lacking GNAS coding mutations and suggest that PHP-Ia patients should be screened not only for GNAS coding mutations but for epigenetic defects at the same locus as well.

P589

Hypospadias and micropenis in congenital adrenal hyperplasia: a case study

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Introduction
Congenital adrenal hyperplasia (CAH) is a group of autosomal recessive diseases with increased adrenal androgens secretion from the adrenal cortex, characterized by simple virilizing and salt wasting forms. Deficiency of 21-hydroxylase, caused by mutations in the 21-hydroxylase gene (*CYP21A2*) is the most frequent CAH, accounting for more than 90 percent of CAH cases. Deficiency of 3 beta-Hydroxysteroid-Dehydrogenase Type II is caused by mutations in the *HSD3B2* gene and accounts for about 1–10 percent of cases of CAH.
Patient
This report is about a 2-year-old patient of Turkish origin referred to our center with clinical finding of penoscrotal hypospadias and micropenis (stretched penile length 1.5 cm). Testicles were palpable bilaterally in the scrotum. Due to initial biochemical and hormonal findings moleculargentic analysis of *CYP21A2* gene was already done, showing heterozygous germline mutations p.Val281Leu, p.Leu307fs, p.Gln318Stop and p.Arg356Trp. His parents are cousin-german to each other.
Methods
Genomic DNA was extracted from peripheral blood leukocytes. Coding regions and corresponding exon-intron boundaries of the *CYP21A2* gene and the *HSD3B2* gene were amplified by PCR and subjected to direct sequencing.
Results
A compound heterozygous state of these mutations was excluded by sequencing analysis of *CYP21A2* genes of both parents (father has no mutation). Further hormonal studies suggested a 3 β-Hydroxysteroid dehydrogenase type II deficiency and justified sequence analysis of the *HSD3B2* gene. A novel homozygous germline mutation (p.Trp355Arg) was found, for which both parents are heterozygous carriers.
Conclusion
To judge a case of CAH in the right way it is important to look at all clinical aspects in a differentiated way. Comprehensive (clinical, biochemical, hormonal) analysis should be conducted and approved by moleculargenetic analysis in line with a genetic counseling.

P590

Increased hyperinsulinism and insulin resistance, and decreased antioxidant defense, in children and adolescents with pre-metabolic versus metabolic syndromes

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Background and aims
The aim was to analyze insulin resistance (IR), glycoregulation disorders, lipid status, C-reactive protein (CRP), plasminogen activator inhibitor (PAI-1) and antioxidant defense in children and adolescents with pre-metabolic (Pre-MS) and metabolic (MS) syndromes.
Material and methods
The study included 30 obese individuals (age 10–20 years, body mass index (BMI) or waist circumference (WC) $\geq$90 percentile). Three of the following five criteria were used for MS diagnosis in children and adolescents: WC $\geq$ 90 Pct. for age and sex; triglycerides (TG) $>$ 1.7 mmol/l; high density lipoprotein cholesterol (HDL-C) $<$ 1.0; hypertension $\geq$90 Pct. for height, age and sex; glycaemia $>$ 6.0. Patients with less than three afore mentioned criteria were indicated as patients with Pre-MS.
Insulin sensitivity was determined by HOMA IR. Serum CRP was measured by immunometric assay. Activities of markers of antioxidant defense, superoxide dismutase (SOD) and glutathion peroxidase (GSH-Px) were determined in erythrocytes.
Results
Metabolic syndrome was found in one third of patients (BMI 33.84 $\pm$ 5.5 kg/m^2; WC 103.9 $\pm$ 15.6 cm; blood pressure 123.8 $\pm$ 11.1/80.0 $\pm$ 9.6 mmHg; increased HOMA IR (5.42 $\pm$ 2.6); increased triglycerides (1.9 $\pm$ 0.6 mmol/l); decreased HDL (0.88 $\pm$ 0.23 mmol/l); increased LDL/HDL ratio (3.5 $\pm$ 1.37); increased CRP (11.64 $\pm$ 15.9 mg/l); increased PAI-1 (5.62 $\pm$ 1.28 U/ml); low SOD and GSH-Px (1001.2 + 117.4 U/grHg and 35.3 + 17.9 U/grHg respectively). The other two thirds showed 1-2 metabolic syndrome criteria (mostly WC 91.6 $\pm$ 16.0 cm; decreased HDL 1.07 $\pm$ 0.16 mmol/l), increased HOMA IR (6.1 $\pm$ 4.4, l), normal CRP (1.4 $\pm$ 2.1 mg/l), increased PAI-1 (3.41 $\pm$ 2.38 U/ml) and decreased antioxidative defense (SOD 932 + 0.81 U/grHg; GSH-Px 31.0 + 6.7 U/grHg).
Conclusion
PAI-1 and proinflammatory cytokines with CRP directly accelerate atherosclerosis and thrombosis. Positive correlations between PAI-1 and WC and BMI, and negative correlations between BMI and antioxidative defense in the pre-metabolic syndrome patients show that this early stage preceding MS is also characterized by evident hyperinsulinism and IR and atherosclerotic complication risks.

P591

Prevalence of obesity and metabolic syndrome among Spanish adolescents

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Purposes
To report the prevalence of obesity, metabolic syndrome (MS) and its related components among adolescents living in the city of Almería (south of Spain). To examine the distribution of HOMA-IR (homeostasis model assessment of insulin resistance).
Methods
A total of 373 subjects attending secundary school (aged 12–17 years) participated in a community-based cross-sectional survey. IOTF (International Obesity Task Force) criteria were used to identify obesity and overweight. Criteria for the MS were the presence of three or more of the following components (criteria for adolescents to Adult Treatment Panel III): 1) central obesity (waist circumference $\geq$90th percentile, age and gender specific; 2) elevated triglyceride concentrations ($\geq$110 mg/dl); 3) low HDL cholesterol concentrations ($\leq$45 mg/dl); elevated blood pressure (systolic and/or diastolic $\geq$90th percentile, age and gender specific); or 5) elevated fasting glucose levels ($\geq$100 mg/dl). HOMA-IR was calculated and linear regression identified factors associated.
Results
About 8.0% of this sample was obese and 20.4% was overweight. The prevalence of MS was 6.2% (95% confidence interval 4.8–7.6). It was 26.7%, 14.5% and 1.5% among adolescents who were obese, overweight and normal weight. 8.3% of the adolescents had two components and 26.0% had one component, with low HDL cholesterol the most common component (19.8%). Mean HOMA-IR was 2.07. Triglyceride concentrations and waist circumference were the most important determinant of HOMA-IR ($\beta = +0.30$; $P < 0{,}001$; $\beta = +023$; $P = 0.013$).
Conclusions
Obesity and MS are major problems for Spanish adolescents. The prevalence of the MS is higher in obese as opposed to non-obese subjects.

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Kinetic study of ldl oxidation in female progeny with positive family history of cardiovascular diseases and/or hyperlipidemia

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Aim
The present study was designed in order to investigate the lipid profile, as well as LDL oxidizability *in vitro*, of female progeny (girls and young women) with positive family history of cardiovascular diseases (CVD) and/or hyperlipidemia.
Patients and methods
In the present study, 30 healthy female subjects were recruited, aged 3 to 30 years old (mean $\pm$ S.D. 14.4 $\pm$ 8.02 years old). These subjects were progeny of families with history of CVD and/or hyperlipidemia. Kinetic study of LDL oxidation with Cu^{2+} took place *in vitro*. Lag time (*t*lag) and malonyldialdehyde (MDA) were used as indexes of LDL oxidizability. Moreover, lipid profile parameters were determined, such as total cholesterol (CH), triglycerides (TG), HDL, LDL, apolipoprotein A1 (apoA1) and B100 (apoB100) and lipoprotein (α) (Lp(α)), after overnight fast.
Results
Mean values $\pm$ S.D. of the parameters under study were: *t*lag 82.16 $\pm$ 16.5 min, MDA 18.19 $\pm$ 4.79 nmol/mg LDL, apoA1 1.39 $\pm$ 0.35 g/l, apoB100 1.16 $\pm$ 0.56 g/l, Lp(α) 24.4 $\pm$ 21.55 g/l. Mean values $\pm$ S.D. of the lipid parameters for subjects $<$16 years old ($n = 18$) and $\geq$16 years old ($n = 12$), were: CH 222.85 $\pm$ 99.2 and 214.58 $\pm$ 45.01 mg/dl, TG 64.2 $\pm$ 23.55 and 93.1 $\pm$ 93.41 mg/dl, HDL 44.1 $\pm$ 9.47 and 47.3 $\pm$ 10.47 mg/dl and LDL 165.9 $\pm$ 95.1 and 149.1 $\pm$ 40.54 mg/dl, respectively. Two thirds of the subjects were of medium or high risk for a future cardiovascular disease or hyperlipidemia. Moreover, *t*lag presented significant positive correlation with HDL serum levels ($r = 0.536$, $P = 0.002$).
Conclusions
In female progeny with family history of CVD and/or hyperlipidemia, lipid profile should be determined for early detection of progeny of high risk for CVD. HDL serum levels should be high above recommended, as long as they seem to be correlated with increased resistance of LDL to oxidation.

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Final height and timing of menarche after treatment for idiopathic central precocious puberty (CPP)

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In true precocious puberty, the increased gonadal steroid secretion increases height velocity, somatic and psychosocial development, and the rate of skeletal maturation and can lead to short adult height. The aim is to assess the impact of suppression therapy of CPP with triptorelin on final adult height and the timing of menarche and pattern of menstrual cycle post-treatment.
Materials and methods
Nineteen girls, with CPP, which had completed at least two years of triptorelin therapy and have attained final height at the time of last evaluation, were incorporated in the study. The diagnosis of PP was based on combined data concerning early occurrence of secondary pubertal signs, advanced bone age (BA) and accelerated growth rate based on multiple height measurements and LHRH response consistent with CPP. Seven girls with central precocious puberty, which did not initiate triptorelin therapy mainly due to their parents' refusal and had attained the final height, were used for comparisons. The mean (S.E.M.) chronological age at diagnosis was: 8.42 (0.15) years and 8.44 (0.57) years and (BA) 10.76 (0.32) and 11.2 (0.43) yrs for patients and controls respectively. Girls were treated with triptorelin (Arvecap, IPSEN, Greece) every 4 weeks at a dose of 3.75 mg.
Results
Mean final height was not statistically different from mean target height (157.28 vs 159.55 cm, $P = 0.055$). Mean age of menarche was statistically different between treated girls and controls (12.45 vs 11.02 years $P = 0.004$). Menarche was observed 1.37 years $\pm$ 0.73 (range: 0.4–3.57) following triptorelin discontinuation. Menstrual cycle was regular ranging from 28 to 40 days in 17 (89.5%) out of 19 girls.
Conclusions
Suppression therapy seems to be effective on attainment of final height in the range of target height. Spontaneous menarche occurs in all patients followed by regular menstruation in the vast majority of treated subjects.

P594

The coincidence of Poland syndrome and Turner syndrome

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Poland syndrome (PS) is a defect consisting in unilateral deficiency of the pectoralis major muscle and anomaly of the ipsilateral upper limb, usually in the form of syndactyly or synbrachydactyly. The absence of a nipple and the aplasia of a mammary gland are frequently found. The incidence of PS ranges from 1:7000 to 1:100 000.
The authors present a 5-year-old girl with a very rare coincidence of PS and Turner syndrome. The child was born after 38 weeks of gestation (weight 2400 g, length 49 cm, head circumference 30 cm, 10 points on Apgar scale) with the feet lymphoedema and the right hand anomaly.
At the age of two a marked difference in height between the girl and her peers was observed. The endocrinological consultation was performed after surgical correction of her hand at the age of 3. On physical examination the following were confirmed: height 83 cm ($<$3 c, $-$2.4 SDS), weight 10 kg ($<$3 c), underdeveloped right pectoralis maior muscle and right upper limb, angular all nails placement, cranial disproportion with prominent forehead, small jaw, gothic palate, lower ear settle, systolic hear murmur, clitoris enlargement, condition after syndactyly operation, deformed elbows and knees.
The bone age was delayed half a year. GH max. during nocturnal profile was 5.91 ng/ml, FSH 38.2 mIU/ml, TSH 1.64 uIU/ml, FT4 1.64 ng/dl, IGF 1 27.7 ng/ml.
On the ground of clinical picture PS was diagnosed and the suspicion of Turner syndrome was suggested. The results of genetic examination confirmed the monosomy of X chromosome. The girl started GH treatment at the age of 4.5.
Poland's anomaly in our patient demands lasting rehabilitation. However, her therapeutic program is more complex because of the coincidence with Turner syndrome.

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Day profiles of salivary 17-hydroxyprogesterone for the control of glucocorticoid therapy in adolescents with congenital adrenal hyperplasia due to 21-hydroxylase deficiency

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Introduction
Optimizing glucocorticoid (GC) therapy in patients with congenital adrenal hyperplasia (CAH) remains a challenge. While overdosing may result in Cushing's syndrome, underdosing is associated with female virilization and adrenal insufficiency. This study evaluated day-profiling of salivary 17-hydroxyprogesterone (17OHP) for the biochemical control of adolescents with CAH due to 21-hydroxylase deficiency.
Methods
Twelve patients (4 males; age 19.3±0.6; BMI 28.0±1.9) were enrolled after transfer from pediatric to adult care. Sampling was performed at 0700, 0930, 1200, 1500, 1800, 2200, and 2400 h. 43 healthy volunteers (22 males; age 37.8±1.8; BMI 25.7±0.7) served as a control group, and upper normal 17OHP cutoffs (mean+2S.D.) were calculated for each time point. Results are expressed as mean±S.E.M. 17OHP was measured by RIA (DPC Diagnostic Products Corp).
Results
Initial evaluation revealed elevated 17OHP baseline values in 64% of patients. Five patients were tested only once (1.8±0.6 sampling times elevated, mean HC-equivalent dose 33.3±10.3 mg), while 7 patients were repeatedly tested. For the latter, 6.0±0.4 sampling times were elevated in the initial day profiles (mean HC-equivalent dose 25.0±1.1 mg). In contrast, 2.9±1.0 elevated sampling times were found after adaption of medication, such as addition of longer-acting glucocorticoids (mean HC-equivalent dose 24.1±2.0 mg). With respect to all profiles, results of single morning evaluation and day-sampling were similar in 38%. Seventy eight percent of profiles with normal 17OHP levels at 0700 h demonstrated increased 17OHP throughout the day.
Conclusion
A relevant proportion of patients with CAH demonstrated insufficient biochemical control during adolescence. Salivary 17OHP day profiles led to changes in GC application with improved 17OHP suppression, while daily GC dose remained stable. Determination of single morning 17OHP levels did not allow for reliable control of androgen excess.

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The superoxide dismutase and lipid peroxide in children with Down syndrome and congenital heart disease

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Metabolic disturbances occur more often in patients with Down Syndrome (DS, trisomy 21) than in the health population.
Superoxide dismutase (SOD-1), the main enzyme in the antioxidative system, is coded on chromosome 21. Disturbances in the antioxidative system may play a major role in the development of complications of CHD with arterio-venous shunt.
The aim was to evaluate the activity of SOD-1 and concentration of LPO in children with DS and CHD.
Sixty-two children with DS (mean 6.7 years) were divided for 3 groups:without CHD (DS-0, $n=18$/CG-0, $n=13$); CHD with insignificant arterio-venous shunt (DS-1, $n=26$/CG-1, $n=26$) and CHD with significant arterio-venous shunt ± pulmonary hypertension (HP) (DS-2, $n=18$ / CG-2, $n=14$).) As a control group were 53 healthy children (mean 10.4 years) as a control group (CG).
The activity of SOD-1 and concentration of LPO were evaluated.
The activity of SOD-1 was statistically higher in DS than in CG group, which is genetically determined, but only in children without CHD (DS-0 vs CG-0: 0.0441 U/mg protein vs 0.0319 U/mg protein, $P< 0.05$).
SOD-1 was higher in CG2 -children than in CG0- children, as well DS as CG. (CG-2 vs CG-0: 0.0608 vs 0.0319 and DS-2 vs DS-0: 0.0568 vs 0.0441; $P<0.05$).
In CG2 children the activity of SOD-1 was higher even in CG than in DS (CG-2 vs DS-2: 0.0608 vs 0.0568).
The level of LPO in all DS groups was lower than in CG groups (DS-0= 1.57 nmol/ml vs CG-0=1.83; DS-1=1.46 vs CG-1=1.58; DS-2=1.41 vs CG-2=1.44).
Conclusions
The high activity of SOD-1 in children with CHD with arterio-venous shunt (as well DS as CG) indicates a disturbances in the antioxidative system in this children.
The higher activity of SOD-1 may play an important role in lipid peroxidation process, which express lower LPO concentrations.

P597

New clinical features and detailed genetic analysis of heterozygous 17q12 deletion syndrome, leading to loss of TCF2 and MODY5

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Objective
MODY5 is caused by abnormalities in the *TCF2* gene encoding the transcription factor HNF1β. We investigated cases of MODY5 for the underlying type of *TCF2* anomaly.
Case presentations
From 623 children and adolescents with diabetes mellitus followed at our diabetes clinic in 2006, 64 were negative for islet cell autoantibodies (GAD, IA-2, ICA) within the first year of diagnosis and out of these, four patients presented clinical features of MODY5. These features were in all patients: renal disease (single cysts, cystic dysplasia or uretero-pelvic obstructions), elevated liver enzymes, reduced birth weight and reduced postnatal growth as well as diabetes with adolescent onset. Additional features in some patients were pancreas dysplasia and exocrine insufficiency (2/4), genital malformation (3/4), and mental retardation (2/4). Single cases presented also extreme pre- and postnatal growth deficit, congenital cholestasis with bile duct dysplasia, immune defect (CVID) or cataract at diabetes onset.
Results
DNA of the patients was analysed for *TCF2* point mutations and if normal by QMPSF for gene deletions. All MODY5 patients found in our cohort had no *TCF2* mutation but had monoallelic loss of the entire *TCF2* gene. Array CGH and FISH analysis confirmed a large genomic deletion of ~1.5–1.7 Mb in size that was not detected in any of the patients' parents.
Conclusion
The phenotype of patients with 17q12 deletion syndrome is highly variable. Up to now, 17q12 deletion syndrome was claimed to be one of the few contiguous gene deletion syndromes without mental retardation but our two cases with mental retardation question this hypothesis. The molecular defect identified in all cases was a 1.5–1.7 Mb deletion and paired, segmental duplications along with breakpoints were most likely involved in this recurrent chromosomal microdeletion.

P598

Aspects and features of type 2 diabetes and the metabolic syndrome in obese children and adolescents

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Background
Obesity is a rising problem in developed and developing countries. Currently little is known about prevalence and prognosis of type 2 diabetes and the metabolic syndrome in obese children and adolescents in Europe.
Patients and methods
About 491 obese children (mean-age 11.2 years; mean-BMI 30.3 kg/m^2) were examined (lipid-profile; blood pressure, insulin-resistance[1]) and in 102 of them

with risk factors for type 2 diabetes (type 2 DM) according to the ADA-criteria (positive family history, acanthosis, ethnic risk group) two oral glucose tolerance tests (OGTT) were performed with 9–12 months interval. The influence of puberty, BMI and therapy was verified by a multivariate analysis.
Results
Hypertension (2nd Task Force) was present in 18.3%, hyperlipidemia (total-cholesterol >200 mg/dl, triglycerides >150 mg/dl) in 27.7% and insulin-resistance (R-HOMA>3) in 46.3%. Overall in 64% of the patients at least one sign of a metabolic syndrome was present. In the diabetes-risk group the OGTT revealed impaired glucose tolerance (IGT) in 36% and type 2 DM in 8% of the patients (all white Caucasian). Longitudinally insulin-resistance was strongly influenced by progression of puberty (Odds-ratio 7.80; $P=0.009$) and increase of BMI (Odds-ratio 3.25; $P=0.031$). Participation on an obesity-training-programme improved insulin-resistance independent from BMI-change (Odds-ratio 4.4; $P=0.008$).
Conclusions
The metabolic syndrome is not only a feature of obesity in adults but also very frequent in children and adolescents mirroring the dimension of childhood obesity and related complications. Type 2 diabetes mellitus in obese children and adolescents is no longer restricted to minority groups. Dyslipidemia, reduced insulin sensitivity and impaired glucose tolerance are closely correlated. Longitudinal studies are needed to establish reliable screening criteria and non-pharmacological and pharmacological prevention strategies for obese children and adolescents.
Matthews DR *et al*. *Diabetologia* 1985 **28** 412–9.

P599

Autoimmune polyendocrine syndrome type 1 in West Northern France: phenotypic and genotypic description, and use of immunosuppressive therapies

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Autoimmune polyendocrine syndrome type 1 (APS1) is an autosomic recessive disease due to *AIRE* gene mutations, inducing central immune tolerance breakdown. It was poorly known in France.
Objectives
To describe clinical and immunological phenotypes, to determine main genotypes in West Northern France (9 millions inhabitants), to identify factors that could influence phenotypes, and to analyse immunosuppressive therapies indications in APS1.
Methods
Clinical and immunological datas have been collected thanks to West Northern France endocrinologists and paediatricians collaboration. Pathological mutations were identified by *DNA* sequencing.
Results
About 19 patients (7 females, 12 males, age 31 ± 13 yr) from 13 families have been identified. Clinical manifestations varied greatly among patients, from 1 to 10 components. Candidiasis and adrenal insufficiency frequently occurred in respectively 17 patients (89%) and 15 patients (79%). Surprisingly, hypoparathyroidism was diagnosed in only 12 cases (63%), whereas alopecia was particularly frequent, in 10 patients (53%). In 3 patients presenting with atypical forms, molecular diagnosis confirmation was essential. Four different *AIRE* gene mutations were identified, and 13bp-deletion in exon 8 (c.967-979del13) was the most prevalent. A correlation between this mutation on at least one allele and alopecia occurrence was identified ($P=0.003$). There was an elevation of mean CD4+ lymphocytes concentrations ($1103\pm256/mm^3$) while mean CD8+ ($412\pm156/mm^3$) and CD20+($174\pm131/mm^3$) lymphocytes concentrations were diminished, with a mean elevated CD4/CD8 ratio (2.51 ± 1.24). Four patients were treated by immunosuppressive therapies: 2 for hepatitis, one for severe malabsorption and one for a T-cell large granular lymphocytes leukaemia. Those therapies were very efficient but a patient deceased of septicaemia (*C. Albicans*).
Conclusion
APS1 frequency in West Northern France was about 1/500 000. Phenotypes varied greatly, and molecular diagnosis was determinant in atypical cases. Immunosuppressive therapies remain essential in severe manifestations, but their initiation should be preceded by candidiasis and other infections careful research and treatment.

P600

High incidence of obesity and insulin resistance in prepubertal children, born too small for their gestational age

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Fetal undernutrition is at the base of disorders in the differentiation process of pancreatic beta cells. Abnormal insulin activity leads to restriction of fetal growth and to obesity, insulin resistance and diabetes mellitus type 2 (DM2), either in childhood or adulthood. The aim of the study was to assess the incidence of obesity and insulin resistance in prepubertal children, born with body mass too small for their gestational age (SGA).
Material and methods
Sixty-eight (68) prepubertal children (aged form 6 to 10 years, born as SGA (the birth weight below -2.0 standard deviation for gestational age and sex), were enrolled into the study. The comparison group comprised twelve (12) healthy children, matched for age and sex, born with body weight appropriate for gestational age (AGA). In each child, its actual height and weight were measured and BMI was calculated, fasting cholesterol and triglycerides concentrations were assessed and glucose and insulin concentrations during oral glucose tolerance test (OGTT) were performed. Based on the results of OGTT, IR index, according to HOMA, and IR index, according to Belfiore, were calculated, too.
Results
Neither glucose intolerance nor DM2 was observed in any of the examined children. No lipid concentration disorders were found in any of the children. Obesity was recognized in 32.3% children with SGA. In about 50% of the children with SGA and obesity, insulin resistance was identified. No statistical correlation was observed between birth body mass and: glucose, insulin, cholesterol and triglycerides concentrations, however, a negative correlation was noted between birth body mass and IR indices.
Conclusion
Low birth body mass is a risk factor of obesity and insulin resistance in prepubertal children.

P601

Prolactinomas in pediatric age

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Introduction
Prolactinomas are the most common pituitary adenomas in paediatric patients, except in the first decade of life, when ACTH secreting adenomas are more frequent.
Objective
Analysis of clinical, diagnostic and therapeutic data of prolactinomas in paediatric age.
Methods
Retrospective study of 15 patients whose symptoms began before 18 years of age.
Results

*Mean $\pm$ S.D.	Macro	Micro
No of boys/girls	3/4	0/8
Age at beginning of symptoms* (year)	15.4 ± 1.9	15 ± 2.4
Age at diagnosis* (year)	18 ± 2.9	18.8 ± 6.4
Basal prolactin levels	35.7 ± 40.2	4.2 ± 1.6
*(elevation above reference)		

In girls, secondary amenorrhea (58.3%) and galactorrhea (41.7%) raised diagnostic investigation whereas in boys (all macroadenomas) this was made by mass effect symptoms. All patients were treated with dopamine agonists and one was submitted to transsphenoidal surgery. Nowadays, 8 have normal prolactin levels, 2 without therapy; the remaining have high levels but are asymptomatic.

Discussion
Most cases were diagnosed after 18 years of age, with a mean of 3 years between the beginning of symptoms and diagnosis. In females, menstrual irregularities and galactorrhea may have led to a precocious investigation and higher prevalence of microadenomas. In males, mass effects symptoms caused by larger lesions prevailed since endocrine symptoms are insidious and more difficult to notice. Treatment with dopamine agonists was effective and without major side effects.
Conclusion
Prolactinomas should always be considered in the evaluation of menstrual irregularities in girls or pubertal delay in both sexes. They assume special importance because of good outcomes with medical treatment in normalizing prolactin levels and reducing tumour size.

P602

Molecular genetic analysis of a patient with hyperinsulinism and deafness

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Congenital hypoglycemic hyperinsulinemia (CHI) is a clinical and genetic heterogeneous entity. Clinical manifestations can vary from serious life threatening to milder difficultly identifiable cases. Children who do not react adequate to medical treatment are subject to pancreatic recession. The molecular ethiology are from recessive mutations of the *ABCC8* (SUR1) and *KCNJ11* (Kir6.2) to dominant mutations of the *GCK* or *GDH* genes. Focal dysplasia characterised by loos of maternal Chromosome 11 and hereby *ABCC8* and *KCNJ11* is a common cause of CHI. In some studies mutations in the *ABCC8* promotor have been shown to cause CHI. In approximately 50% of the incidences the disease is still genetically unexplained necessitating the search for other genetic factors.
ABCC8 and *KCNJ11* is localised to chromosome 11p15. Interestingly the USH1C gene is localised upstream of *ABCC8*. Usher syndrome type I caused by mutations in *USH1C* is an autosomal recessive sensory defect involving congenital profound sensorineural deafness, vestibular dysfunction, and blindness due to progressive retinitis pigmentosa.
The adjacent *USH1c* gene and *ABCC8* gene of chromosome 11p15 were analysed using quantitative realtime PCR and microsatellite markers to analyse for Loss of heterozygosity (LOH), and sequencing.
The microsatellite D11S902 was absent. By PCR of *ABCC8* it was shown that only exon 23* to 39 are present. All of *KCNJ11* is present. In the same way only exon 1 and 2 of the *USH1c* gene was shown to be present. By sequencing, a homozygous contiguous partial gene deletion was identified, starting *in USH1c* intron 2, c.90+592, and ending in *ABCC8* intron 21.
We here report the analysis of a patient with a complex phenotype that can be explained by a large deletion involving two genes.

P603

Insipidus diabetes revealing a chordomas of a skull base in a child

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Introduction
Chordomas are rare tumours that usually occur in adults, in children since 1923, <100 cases has been reported. It represent few than 5% of these tumours and most frequently develop in the skull base.
Para cellar localisation is uncommon, cause hypopituitarism and oculomotor nerves palsy.
Chordomas are believed to behave more aggressively than chordomas in adults.
Case report
A 14-year-old boy presented in December 2006 with polyuropolydipsia syndrome and headache.
1-On examination: he had
- Short stature (< −2s.D).
- Hypothyroidism signs.
- Pubertal stage G1P1 of Tanner.
- Left oculomotor nerves palsy.
- Ophthalmologic evaluation: normal visual field.

2-Serum hormonal investigation: found hypopiytuitarism and insipidus diabetes.
3-MR imaging demonstrated a mass developing in the clivial region invades the cavernous sinus, the para sellar and the temporal regions. A trans temporal surgery removed apart of the tumour.
4-Pathological findings: physalliphorus cells arranged in lobules and embedded in a mucoid stroma
5-Post operative course was uneventful, two months later the tumour had growth explosively, became exophytic behind the temporal region, and the patient died.
Conclusion
Chordomas is uncommon tumours in child in the case report the insipidus diabetes have revealed them. The para sellar localisation explains the hypopituitarism. The explosively evolution may be explained by a sarcomatous transformation or the expression of growth factors: TGF alpha, BFGF and the strong fibronectine.

Reproduction

P604

Age-adjusted variations in dynamics of the follicular growth in relation to ovarian steroid hormones in the domestic hen

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In birds similar to mammalian species, the reproductive performance in females deteriorates with age. In the present study, relationships between the growth of preovulatory follicles (PF), reproductive aging and sex steroids were investigated using young hens with long clutch (YLC), old hens with long clutch (OLC) and old hens with short clutch (OSC). 1) hens were killed 1.5 and 14.5 h after assumed ovulation. 2) YLC hens were killed 3.5 h after treatments with LH and/or aminoglutethimide (AG), an inhibitor of steroid synthesis. Volumes of PF (F1-F5) and plasma concentrations of ovarian steroids were determined. 3) Granulosa (GC) and theca cells (TC) from F3 follicles of YLC and OSC hens were exposed *in vitro* to estradiol-17beta (E_2, 1 ng/ml) and the proliferative activity (PA) of the cells was examined using CellTiter 96 Aqueous One Solution Assay. In YLC and OLC groups, the total follicular volume (TV) rose between 1.5 and 14.5 h after ovulation (YLC: from 31.8±1.3 to 43.2±2.6 ml; OLC: from 35.7±2.0 to 46.4±2.5 ml, $P<0.01$), negatively correlating with the plasma level of E_2 ($P<0.01$). By contrast, there was no growth of PF in the middle of the ovulatory cycle in the OSC group, with a positive correlation being present between E_2 and TV ($P<0.05$). In young hens, AG caused a decline in levels of steroids and a rise in TV, which was associated with a fall in E_2 ($r=-0.54$, $P<0.05$). E_2 increased *in vitro* the rate of viable GC (YLC: from 201 to 250%; OSC: from 175 to 210%, $P<0.01$) and did not affect PA of TC from both groups. E_2 seems to play a dual role; it stimulates the follicular growth in old hens, whereas it may inhibit the growth of PF in young animals.

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The relationship between semen parameters and blood hormone in men with congenital absence of vas deferens

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Aim
To investigate the ralationship between semen parameters and serum follicale-stimulating hormone (FSH), luteinizing hormone (LH), testosterone (T), prolactin (PRL) in a group of Chinese men with congenital absence of vas deferens (CAVD).
Methods
Ninety patients with CAVD were investigated. During the physical examination the body hair distribution, testicular size, epididymides and vas deferens were carefully inspected and palpated. Semen analysis including liquefy, volume, pH were done. Blood hormone of FSH, LH, T and PRL from 47 cases was detected. The relationship between semen parameters and blood hormone was analyzed by SPSS programe.
Results
In all of 90 patients there were a normal body hair distribution. The testicular size was 16.17±4.07 ml. Seventy-six (76/90, 84.44%) cases with bilateral absence of vas deferens and 14 (14/90, 15.56%) cases with unilateral absence (3 for the left and 11 for the right) were found. There was a normal liquefy time (17.72± 5.34 min), lower semen volume (0.71±0.38 ml) and lower pH (6.47±0.32).

There was a normal FSH, LH, T and PRL level in most of patients except elevated FSH, LH, T, PRL in 2 (4.26%), 9 (19.15%), 2 (4.26%), 7 (14.89%) and lower T, PRL in 4 (8.51%), 5 (10.64%), respectively. There was a positive correlation between FSH and testicular volume ($r=0.367$), liquefying time ($r=0.307$), semen pH ($r=0.291$), LH and semen pH ($r=0.312$). A negative correlation between PRL and semen volume ($r=-0.313$) was found.
Conclusion
In the azoospermia men with lower semen volume, low semen pH and normal liquefying time the clinical diagnosis of CAVD should be suggested. It is importance for the FSH detected in the patients with CADV. To constitute the distinction between CADV and other obstructive azoospermia, for example, vasectomy, the results of semen volume and semen pH may be useful parameters.

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Pituitary primary cell culture and effect of its secretion on endocrine activity of incubated ovarian follicles (Study of pituitary–ovary axis): in the model of fish (common carp)

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In this study, pituitary of five carp fish were collected. The pituitary cells were dispersed enzymatically and cultivated as monolayers in MEM for 72 h. Then the culture media were collected and frozen in -20 °C.
The carp ovaries were also collected and their follicles were separated mechanically and incubated in BSS Cortland medium in 24 well microplates for 48 h in room temperature. Then the incubated ovarian follicles were divided in two groups:
A. Control group which were incubated in BSS medium.
B. Experimental group which divided to three subgroups according to treated with different concentration of collected pituitary medium (50, 100, 200 µl/ml).
Follicles culture media were collected after 72 h and were analyzed for 17-β-Oestradiol (E_2) and 17-α-Hydroxy progestron (P_4) content by radioimmunoassay (RIA).
The results showed that the steroid hormones (E_2 and P_4) secretion in incubated ovarian follicles were increased significantly by low concentration (50 µl/ml)of pituitary media (respectively $P<0.001$ and $P<0.05$) but the high concentration (200 µl/ml) decreased the secretion of E_2 and P_4 significantly (respectively $P<0.01$ and $P<0.05$).
The concentration of 100 µl/ml of pituitary culture media had not affected on steroid hormones secretion significantly.
The interesting finding is that pituitary culture media (which content gonadotropin hormones) were stimulated ovarian follicles for steroid secretion *in vitro*, whereas the high concentration of pituitary secretion decreased the responsiveness of the ovarian follicles which it may occurred due to down – regulation of the pituitary hormone receptors on the ovarian follicles.

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Serum stable metabolites of nitric oxide (NO_x) concentrations in pre- and post-menopausal women on transdermal estradiol therapy

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Since estrogens are known to have some antiatherosclerotic properties and influence on endothelial function, including nitric oxide (NO) synthesis, the purpose of this study was to determine NO_x levels after menopause and to find whether the administration of estrogen therapy restores plasma NO_x levels back to normal. The study group consisted of 26 women with surgically induced menopause, mean age 50.9 ± 2.9 years, and the controls were 40 healthy pre-menopausal women, mean age 48.3 ± 2.3 years. Post-menopausal women were treated for four months with transdermal estradiol. Blood samples were collected for estimation of E_2, FSH, NO_x and lipid profile before and after therapy. Serum NO_x concentration in the study group was significantly lower than in controls ($P<0.01$) and it increased after treatment ($P<0.01$) reaching the values observed in the controls. The statistical analysis showed significant correlation between NO_x concentration and E_2 concentration in menopausal group before ($r=0.25$, $P<0.05$) and after ($r=0.46$, $P<0.001$) treatment. TC, LDL-C, TG, and azpo-B levels were higher in postmenopausal group comparing to controls, and after estrogen therapy they decreased to the values observed in the control group. HDL-C and its subfractions were lower in postmenopausal women than in controls and they improved after hormonal therapy. The same tendency was observed concerning apo-A1. There were no correlations between NO_x and lipids or apolipoproteins. We conclude that low E_2 levels in postmenopausal women were associated with lower NO_x levels. Estrogen therapy improves NO synthesis and relaxation of the vessels.

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Expression of ghrelin receptor, GHSR-1a in the pig ovary and its role in ovarian function

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Ghrelin, a 28 amino acid peptide, was recently isolated from the stomach of rat, and was identified as the endogenous ligand for the growth hormone secretagogue receptor, GHSR. Two GHSR subtypes, generated by an alternative splicing of a single gene, have been identified: the full-length type 1a receptor and truncated type 1b. The GHS-R1a is the functionally active, signal transduction form of the receptor. Ghrelin is recognized as an important regulator of growth hormone secretion (GH), food intake and a factor which controls reproduction. In addition, ghrelin and GHSR-1a expression were detected in reproductive tissue: placenta, ovary and testis. Recent date has suggested that ghrelin may also have an effect on sex hormone secretion and cell apoptosis. The aim of the study was analyze 1). Expression of GHSR-1a in ovarian follicles and the effect of GH on GHSR-1a expression; 2). Involvement of GHSR-1a in estradiol secretion, aromatase activity and caspase -3 activity. Pig ovarian follicles (3–4 mm) were collected from prepubertal animals. To examined the expression of GHSR-1a and influence of GH on GHSR-1a expression we used Western Blott and RT-PCR methods. Using antagonist of ghrelin receptor (D-Lys-3)-GHRP-6 (50 ng/ml), involvement of GHSR-1a in estradiol secretion, aromatase activity and caspase-3 activity were examined. Estradiol concentration was measured using direct enzyme immunoassay. Aromatase activity was measured using fluorometric substrate and caspase-3 activity colorimetric substrate. We demonstrated presence of GHSR-1a in prepubertal pig ovary and no influence of GH on both GHSR-1a protein and mRNA expression. Moreover, our study confirmed the involvement of GHSR-1a receptor in estradiol secretion and aromatase activity but not in cells apoptosis. In conclusion, our study provides the novel evidence for the expression of the GHSR-1a in the pig ovary and suggest involvement of this receptor in the control of key ovary function.

P609

A comparison between flutamide and finasteride plus flutamide combination in the treatment of hirsutism

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Objective
To investigate the clinical efficacy and safety of the finasteride (5 mg/day) plus flutamide (125 mg/day) combination therapy in unselected women with moderate to severe hirsutism.
Patients and methods
Thirty hirsute women were randomly assigned to two treatment groups. Sixteen patients (group 1) were treated with flutamide (125 mg per day) and 14 patients (group 2) were treated with finasteride (5 mg per day) plus flutamide (125 mg per day) for 12 months. Hirsutism score was measured according to the modified Ferriman–Gallwey scoring system. Pre and post-treatment evaluation of hirsutism score, and serum FSH, LH, estradiol, total testosterone, free testosterone, androstenedione, DHEAS and SHBG were obtained. Blood chemistry and side effects were evaluated during the study.
Results
At the sixth month of the treatment, flutamide alone and combination therapy resulted in similar improvements. The reductions in hirsutism score (% of the baseline) at 6 months were 35% for group 1 and 33% for group 2. Combination therapy also resulted in (49%) similar improvement to flutamide alone (45%) at 12th months of the treatment.
Conclusion
Although both drugs have different action mechanisms, combination of flutamide and finasteride is not better than flutamide alone in women with hirsutism.

P610

Quantitative determination of aromatase and 5-α reductase mRNA and polymorphisms in the aromatase and 5-α reductase genes in idiopathic hirsutism

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Objective
Hirsutism, affects between 5% and 10% of women during reproductive age. Hirsutism associated with normal ovulatory function and normal circulating androgen concentrations is defined as idiopathic hirsutism and little information is available regarding the molecular pathogenesis.
Aim
Significance of polymorphisms of the genes involved in androgen/oestrogen biosynthesis (*SRD5A2,CYP19*) and their expressions inmodulating the susceptibility to idiopathic hirsutism were studied.
Methods
Hirsutism was scored using a modified Ferriman–Gallwey scoring system. The score >8 was considered as hirsutism. All the patients had idiopathic hirsutism. We assessed the expression of the genes for type 2 (SDR5A2) 5-alpha reductase isoenzyme and aromase (CYP19) in hair follicle removed from midline subumblical region in 8 untreated idiopathic hirsute patients and 8 normal women. SDR5A2 and CYP19 expression were estimated by Real-Time-PCR using the gene of the ubiquitously expressed protein β-actin as an internal control.
Results
No differences were found in SDR5A2 expression levels between patients and normal women. CYP19 gene expression levels were lower in the patients when compared with the controls ($P<0.05$).
Conclusion
Decreased aromatase activity may contribute to the pathogenesis of idiopathic hirsutism.
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Endometriotic cell proliferation and survival are inhibited by somatostatin analogues and GHRH antagonists

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Somatostatin (SST) and growth hormone-releasing hormone (GHRH) are hypothalamic hormones that, respectively, inhibit and stimulate pituitary GH secretion. In addition, in peripheral tissues somatostatin and its analogues inhibit, whereas GHRH stimulates, both normal and cancer cell growth and survival. Furthermore, both somatostatin receptor 2 (SSTR-2) and GHRH expression have been shown in normal human endometrium. Endometriosis is a common estrogen-dependent disorder defined by the presence of endometrial cells outside the uterus, resulting in pelvic pain and infertility. With respect to eutopic endometrium, the ectopic shows higher local oestradiol bioavailability, abnormalities in gene expression, impaired sensitivity to apoptosis and increased cell proliferation.
Aim of this study was to investigate the expression of GHRH and SST receptors in ectopic endometrium of patients with endometriosis and to test whether SST analogues and GHRH antagonists would inhibit *in vitro* proliferation and survival of primary endometrial cells obtained from endometriotic implants. In ectopic endometria ($n=18$) RT-PCR experiments showed the expression of all five somatostatin receptor subtypes (SSTR1 to 5), and of the splice variant-1 (SV1) of the GHRH-receptor (GHRH-R). By Real-time PCR we found that in 64% of patients SSTR2 and SSTR5 were significantly more expressed in ectopic than in eutopic tissues. Importantly, the SST analogues Lanreotide and Octreotide dose-dependently reduced ectopic more than eutopic endometrial cell growth and survival. Noteworthy, GHRH antagonist JV-1-36 showed significant inhibitory effect on stromal endometrial cell proliferation and viability.
In conclusion, these findings suggest that SST analogues, as well as GHRH antagonists, may be useful molecules for inhibiting proliferation and survival of ectopic endometrial cells in patients with endometriosis.

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Developmental and genetic changes in chemosensory activation of testicular testosterone response to a receptive female and sexual behaviour in laboratory male mice

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Chemosensory cues from receptive females produce the activation of the pituitary–testicular axis and facilitate a complex display of sexual behavior in male mice. Testosterone output in response to a receptive female and the pattern of sexual behaviour were investigated in male mice ($n=190$) of three inbred strains BALB/cLac, CBA/Lac and PT at puberty (45 days of age) and in adulthood (90 days). Sexually naive pubertal or adult male was exposed for 10 min to a receptive female separated by a plastic grill, which would not allow contact between them. Male behaviour was recorded by measuring the time the male spent at the grill and the number of approaches to it (sexual motivation). The grill was then removed and sexual behavior (the number of mounts, nasal and anogenital sniffing) was recorded for next 20 min. An increase in serum concentration and testicular content of testosterone was used as a reflexive endocrine index of the sensitivity to female pheromones. Testosterone was determined by the competition enzyme immunoassay. It has been shown significant effects of developmental and genetic factors on the testosterone response to sexual stimuli and sexual behaviour. The pubertal BALB/cLac males were characterised by the adult pattern of precopulatory behaviour and the evident testosterone respond to a female. The PT males showed the lowest level of behaviour towards a female and no testosterone response at both ages. The CBA/Lac's demonstrated the developmental increase in endocrine responses and sexual behaviour, the highest number of mounts and the moderate testosterone respond to a female at adulthood. The patterns of sexual behavior and testosterone response to a receptive female in three inbred mouse strains demonstrate the genotype-related maturation of the pituitary–gonadal axis and neural circuits of sexual behavior, and provide confirmation that genetic differences are a major source of variation in reproductive maturation.

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Growth hormone improves semen volume, sperm count and motility in men with idiopathic normogonadotropic infertility

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Objective
The aim of this exploratory study was to assess the effect of growth hormone on semen parameters in men with primary infertility and normogonadotropic idiopathic oligoasthenospermia.
Method
This study was performed as a prospective, open- label, non-randomized observational study in a private practice in fourteen men, aged 26 to 35, with normogonadotropic idiopathic oligoasthenospermia. Growth hormone 1.5 IU/day was administered for 6 months and semen parameters assessed on a monthly basis. All were normogonadotropic, with normal sexual function, normal body hair pattern and masculinization, normal testicular volume and penile size. All had received various treatments for infertility in the past, including successful treatment for pyospermia in seven of the cases. Fine needle aspiration cytology revealed normal to – depressed spermatogenesis, with cells of all stages visible, in all patients.
Results
The initial sperm count varied from 0.1 to 10 million/ml, with motility ranging from 1 to 50%. Semen volume, count and motility improved in all patients. Volume increased from 0.63 ± 0.40 ml to 1.24 ± 0.42 ml ($Z=3.68$; $P<0.01$), count from 3.76 ± 3.42 to 5.87 ± 6.76 million/ml ($Z=1.04$; $P=0.05$), and motility index from $19\pm11.67\%$ to $24\pm12.1\%$ ($Z=1.11$; $P>0.05$). The increase was most marked during the first three months of therapy. Not much improvement was noticed during the later half of treatment. None of the patients experienced any side effects. Three subjects fathered children over the next 1 year, two with the help of intrauterine insemination.
Conclusion
Growth hormone can be explored as an effective treatment for men with primary infertility and normogonadotropic idiopathic oligoasthenospermia.

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Expression and direct effects of adiponectin in rat testis

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The adipose tissue is an active endocrine organ involved in the control not only of metabolism and energy balance, but also of other relevant body functions, including reproduction. Adiponectin is an adipocyte hormone, with relevant roles in lipid metabolism and glucose homeostasis, recently involved in the control of different endocrine organs, such as the placenta, pituitary and, likely, the ovary. However, whether as described previously for other adipokines, such as leptin and resistin, adiponectin is expressed and/or conducts biological actions in the male gonad remains unexplored. In this study, we provide compelling evidence for the expression, putative hormonal regulation and direct effects of adiponectin in the rat testis. Testicular expression of adiponectin was demonstrated along postnatal development, with a distinctive pattern of RNA transcripts and discernible protein levels that appeared mostly located at interstitial Leydig cells. Testicular levels of adiponectin mRNA were marginally regulated by pituitary gonadotropins, but overtly modulated by metabolic signals, such as glucocorticoids, thyroxine and the PPAR-γ ligands. In addition, expression of the genes encoding adiponectin receptor 1 (AdipoR1) and AdipoR2 was detected in rat testis, with developmental changes and gonadotropin regulation for AdipoR2 mRNA, and prominent levels of AdipoR1 in seminiferous tubules. Moreover, recombinant adiponectin significantly inhibited basal and human CG-stimulated testosterone secretion *ex vivo*, while it failed to change relative levels of several Sertoli cell-expressed mRNAs, such as stem cell factor and anti-müllerian hormone. In sum, our data are the first to document the expression, regulation and functional role of adiponectin in rat testis. Taken together with its recently reported expression in the ovary and its effects on luteinizing hormone secretion and ovarian steroidogenesis, these results further substantiate a multi-faceted role of adiponectin in the control of the reproductive axis, which might operate as endocrine integrator linking metabolism and gonadal function.

P615

Association between psychiatric symptoms and erectile dysfunction

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Objectives
Erectile dysfunction (ED) is often associated with a wide array of psychiatric symptoms, although few studies systematically address their specific association with ED determinants. Aim of this study is to explore the relationship between ED and different psychopathological symptoms.
Design and methods
A consecutive series of 1388 (mean age 51 ± 13 years) male patients with ED was studied. Several hormonal and biochemical parameters were investigated, along with SIEDY (13 item Structured Interview, which identifies and quantifies the contribution of organic, relational and intrapsychic domains of ED) and the Middlesex Hospital Questionnaire (a self-reported test for the screening of mental disorders in a non-psychiatric setting).
Results
Psychiatric symptoms resulted differentially associated with SIEDY domains. Depressive and phobic-anxiety symptoms were associated with the relational domain, somatization with the organic one, while free-floating anxiety, obsessive-compulsive and phobic symptoms were significantly related with higher intrapsychic SIEDY scores. In addition, relevant depressive symptomatology (D) was associated with hypogonadism, the presence of low frequency of intercourse, hypoactive sexual desire (HSD), and conflictual relationships within the couple and the family. Patients with high free-floating anxiety symptoms were younger, and complained of an unsatisfactory work and a conflictual relationships within family. Conversely, subjects with higher phobic anxious symptoms displayed a more robust relational functioning. Similar results were observed in subjects with obsessive-compulsive symptoms, who also reported a lower prevalence of HSD. Finally, subjects with somatization symptoms showed the worst erectile function.
Conclusions
The main value of this study is that it alters various clinicians that many psychiatric symptoms can be found among ED patients. Systematic testing of patients with ED, through psychiatric questionnaires, is recommended to detect even slight or moderate psychopathological distresses, which specifically associate and exacerbate sexual disturbances.

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Estimated cardiovascular risk and arteriogenic erectile dysfunction

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Objectives
Recommending general dynamic penile color doppler ultrasound (D-PCDU) screening in patients with erectile dysfunction (ED) has been questioned due to an inadequate cost-benefit ratio. The aim of the present study is to asses the validity of different risk scores in the identification of patients being screened for arteriogenic ED at D-PCDU.
Design and methods
A consecutive series of 738 (mean age 53.6 ± 9.2 years) patients with ED was studied. All patients underwent D-PCDU. Arteriogenic ED was defined when peak systolic velocity (PSV) was lower than 25 cm/s. The assessment of cardiovascular risk was evaluated using different risk engines, derived from the Framingham, the PROCAM and the Progetto Cuore studies. An iterative ROC curve analysis was used to determine the most proper threshold for different scales for the screening of arteriogenic ED. Sensitivity and specificity at those thresholds were calculated.
Results
Among the patients studied, 52 (7%) had PSV $<$ 25 cm/s. The area under the ROC curves for pathological PSV in relation to cardiovascular risk estimated with different engines was 0.762 ± 0.03, 0.716 ± 0.03 and 0.667 ± 0.03 for Progetto Cuore, Framingham and PROCAM engines, respectively. Sensitivity and specificity of Progetto Cuore estimated risk were 67, 71% when a threshold of 15% was chosen. Corresponding figures for Framingham and PROCAM engine were 74, 57% and 69, 55%, respectively.
Conclusions
If D-PCDU is performed only on patients with cardiovascular risk $>$15%, who represent about 1/4 of all patients (26.8%), as estimated by Progetto Cuore, about 70% of cases of arteriogenic ED can be identified. This means that well over two thirds of cases can be diagnosed by performing D-PCDU on one patient out of four. Estimated cardiovascular risk, assessed through risk engines, could be used to identify patients who should undergo D-PCDU evaluation for the diagnosis of arteriogenic ED.

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Anti-Müllerian hormone levels in women with polycystic ovary syndrome before and after metformin therapy

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Recently was established, that in the ovary Anti-Müllerian hormone (AMH) is produced by the granulose cells and correlated with the count of small antral follicles. As AMH is largely expressed throughout folliculogenesis, it is considered that the serum levels of AMH may represent both the quantity and quality of the ovarian follicle pool. To determine the changes of AMH in women with polycystic ovary syndrome (PCOS) we have studied 22 patients with this disorder and compared their results to those of 20 healthy women at the same age without hyperandrogenism. The AMH levels in women with PCOS (42.34 ± 6.42 pmol/l) were significantly elevated in comparison with those of the controls

(21.58 ± 3.41 pmol/l), $P < 0.01$. AMH concentrations in the overweight patients were with 32.16% higher than in the normal weight women, but the difference was not statistically significant. After six months metformin therapy 2550 mg/daily the levels of AMH decreased from 44.84 ± 10.07 to 35.97 ± 7.06 pmol/l, but not significantly, whereas the total testosterone levels reduced significantly from 3.51 ± 0.33 to 2.58 ± 0.26 nmol/l, $P < 0.05$.
Significant inverse correlations of serum levels of AMH with insulin concentrations ($r = -0.452$, $P < 0.05$) as well as with homeostasis model assessment (HOMA) index ($r = -0.613$, $P < 0.05$) were found in the controls.
In conclusion, the serum levels of AMH are increased in women with PCOS and AMH measurement can be used as a marker with high specificity and sensitivity for PCOS.

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Catecholestogens inhibit endothelial cell growth in an angiogenesis bioassay

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Little is known about the negative regulation of angiogenesis in the ovary. Recent evidence has revealed that estradiol 17β and its metabolites may play an important role in this fine tuning[1]. In a previous work[2], we set up an assay to quantify 2-hydroxyestradiol (2-OHE) and 4-hydroxyestradiol (4-OHE) in follicular fuids. In order to get an insight on the angiogenic effect of 4-OHE and 2-OHE, the objective of this research was to study their possible modulatory role on VEGF-induced endothelial cell growth. To this purpose, we set up a reliable bioassay which allows the study of porcine aortic endothelial cells (AOC) growth on a three dimensional fibrin gel matrix[3]. AOC were cultured on microcarries beads and then pipetted into a solution of fibrinogen and thrombine; cells were then treated with VEGF (100 ng/ml) in the presence or absence of 4-OHE or 2-OHE (1, 10 and 100 ng/ml) and incubated at 37 °C under humidified atmosphere (5% CO_2) for 48 h. Endothelial cell proliferation was measured by means of the public domain NIH program Scion Image Beta (Scion Corporation, MA, USA). A significant ($P < 0.001$) inhibition of AOC growth was induced by both catecholestrogens. No differences have been observed among the different concentration tested ($P < 0.001$). These data suggest that 4-OHE and 2-OHE, the endogenous estradiol metabolites present in the ovarian follicle, can potentially act as physiological antiangiogenesis regulators.
This research was supported by a FIL grant.
1. Basini *et al. Steroids* 2007 **72** 660–665.
2. Basini *et al. Reprod Domest Anim* 2007 **42** 211.
3. Basini *et al. Biofactors* 2007 **29** 11–18.

P619

Intima-media tickness of carotid artery in young women with polycystic ovary syndrome

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Background
Polycystic ovary syndrome (PCOS) is a common endocrine-metabolic disease that occurs in about 10% of women in reproductive age. Today, PCOS is considered not only reproductive edocrinopathy but also a metabolic disorder associated with long-term health risks, including diabetes mellitus and cardiovascular disease.
Objectives
The aim of this study was to evaluate the presense of early vascular demage in normal-weight women with polycystic ovary syndrome.
Methods
The study group consisted of 15 normal weight young women with PCOS (BMI 23.4 ± 4.8 kg/m^2, age 25.1 ± 5.4) and 10 healthy controls (BMI 20.7 ± 1.5 kg/m^2, age 26.8 ± 6.5). A complet hormonal assay was performed in each subject. Serum glucose, basal insuline levels, holesterol, HDL, LDL, and triglyceride were measured. Arterial structure was evaluated by intima-media tickness (IMT) of carotid artery which is a morphological marker of precocious atherosclerosis. Longitudinal ultrasonografic scan of the carotid arteries were performed by expirienced ultrasonographer who was blinded to clinical data. IMT was measured from the B-mode screen by 7.5 MHz linear probe of both common carotid arteries. Statistical analyse was done by ANCOVA using cholesterol, HDL, LDL, triglyceride and baseline insulin level as covariable (separatly).
Results
We found statisticaly significant difference between IMT in PCOS women (0.51 ± 0.06 mm) versus healthy controls (0.42 ± 0.03 mm). Only PCOS effect was significant.
Conclusions
In this group of patients, differences in IMT seems to be atributable to PCOS facors other than insulin, cholesterol and trigyceride levels.

P620

Basal INSL3 levels predict LH and androgen response to GnRH analogue in PCOS women.

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In a previous study, we demonstrated that the concept of functional ovarian hyperandrogenism (FOH), as documented by the exaggerated 17OHP response to buserelin (GnRH-analogue) does not represent a specific feature of all women with PCOS, being present in less than half of PCOS and is a condition characterized by more severe hyperandrogenemia, glucose-stimulated B-cell insulin secretion and worse insulin resistance. INSL3 is produced in the Leydig cells and at reduced levels in ovarian thecal cells. We previously found that in PCOS serum INSL3 levels were significantly higher than controls and that in PCOS they were positively correlated with basal LH and 17OHP response to buserelin. We therefore carried out this study in two groups of PCOS characterized by normal (NR, $n = 6$) or high (HR, $n = 6$) 17OHP response to buserelin, and matched controls ($n = 44$) to investigate whether INSL3 circulating levels may differ in PCOS subgroups, GnRH stimulation affects basal INSL3 concentration and whether a relationship exists between basal INSL3 levels and hormone response to buserelin. INSL3 concentratiosn were significantly higher in PCOS respect to controls ($P = 0.001$). Moreover, in HR INSL3 levels were higher than in NR ($P = 0.006$). Within PCOS the levels of INSL3 positively correlated with FAI ($P = 0.013$) and negatively with SHBG ($P = 0.018$). Moreover, a positive correlation with the % increase of LH after 60 min ($P = 0.037$) and with the LH_{AUC} after 60 min ($P = 0.014$) and after 24 h ($P = 0.014$) was found. Finally, INSL3 levels did not change after buserelin stimulation. These data confirm that PCOS women with FOH are more hyperandrogenic that those without it. Moreover, we have found that INSL3 levels may predict ovarian LH and androgen response to buserelin, which further suggests a potential role of INSL3 in the pathophysiology of hyperandrogenism in PCOS.

P621

Semen quality in men with obesity and metabolic syndrome

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Aim
The aim of the study was to compare the semen quality in men with metabolic syndrome/MS/ and healthy controls.
Materials and methods
Semen samples were collected from 35 males (mean age – 27.45 ± 8.00 years). Nineteen of them had the features of the metabolic syndrome according to the IDF definition and 16 were healthy volunteers. The semen samples were analyzed by only one experienced researcher according to the World Health Organization guidelines.
Results
The patients with MS had similar age, ejaculate volume, percentage of spermatozoa with normal morphology, sperm concentration (in million per milliliter), and total sperm count compared to the controls. However, they had higher percentage of non-motile spermatozoa ($P = 0.032$). Men with obesity (BMI ≥ 30) had significantly lower sperm concentration ($P = 0.03$) and total sperm count (0.021) in comparison to normal – or overweight males.
Conclusion
Reduced semen quality could be established in patients with obesity and MS. Further investigations are necessary to clarify the changes in the exocrine testicular function in males with MS and their consequences for the reproduction.

P622

Low-grade chronic inflammation in first-degree relatives of women with polycystic ovary syndrome

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Polycystic ovary syndrome (PCOS) is a common endocrine disorder in women of reproductive age characterized by chronic anovulation and hyperandrogenism, and also associated with insulin resistance. Subclinical chronic inflammation might be an important pathogenetic factor in the development of insulin resistance, type 2 diabetes and recently has been also observed in PCOS. There is evidence for a familial predisposition not only to hormonal abnormalities but also to metabolic disorders in first-degree relatives of women affected by PCOS.

This study was performed to determine whether sisters and brothers of women with PCOS had evidence for a low-grade chronic inflammation and to evaluate the associations between the markers of chronic inflammation and various components of insulin resistance, hormones, and metabolic parameters.

One Hundred women with PCOS, 40 sisters and 40 brothers of these probands were studied. Levels of hormones (T, DHEA-S, androstendione, 17-OHP, LH, FSH, PRL, TSH) SHBG, hsCRP, fibrinogen, WBC count, serum lipid profile, glucose and insulin (at baseline and during OGTT – 0′, 30′, 60′, 120′) were measured. Insulin resistance was assessed by fasting insulin, HOMA-IR, FIRI and area under the curve for insulin during the OGTT.

Insulin resistance, hyperinsulinaemia and decreased SHBG level was common in family members of PCOS women independently of BMI. We observed higher levels of hsCRP, fibrinogen and WBC count in women with PCOS but not in sisters and brothers. Markers of chronic inflammation positively correlated with BMI and insulin resistance.

The probability of finding the metabolic disorders, particularly insulin resistance, in the first-degree relatives of women with PCOS is higher than in the control groups. It appears that a low-grade chronic inflammation is stronger related with central obesity than with PCOS status *per se*.

P623

Pathogenesis of the congenital forms of hypogonadotropic hypogonadism

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In purpose to elucidate pathogenetic mechanisms of congenital forms of hypogonadotropic hypogonadism we have examined 25 women with idiopathic hypogonadotropic hypogonadism (IHH), mean age 26 years and 7 months (group 1). All patients had amenorrhea, little breast development (Tanner 2–3), absence or little axillery and pubic hair, infantile uterus and decreased ovary volume. There were no pathological changes during MRI-investigation. Twenty healthy women were included in control group (group 2).

Six patients had no significant difference in serum gonadotropins compared with control group (LH 4.7 ± 2.4 U/l; FSH 5.2 ± 1.2 U/l versus LH 5.43 ± 0.57 U/l; FSH 5.63 ± 0.31 U/l respectively). Peaks of LH and FSH every 10 min during 4 h were measured. There was a significant difference of basal LH and FSH concentration in patients with IHH and in the control group (LH 3.7 ± 3.1 vs 6.9 ± 3.1 $P < 0.05$; FSH 5.2 ± 1.8 vs 7.5 ± 1.7 $P < 0.05$). The significant decrease in the gonadotropin oscillations was found on the scattering diagrams comparing to the control group.

The genetic study showed polymorphism in gene of receptor to GnRH (Ser151Ser (AGC-AGT) and in β-subunit of FSH gene another polymorphism was found (Y76Y). These changes were previously described in literature and did not lead to the amino acid replacement.

The presence of antipituitary autoantibodies was detected in 8 (33.3%) patients with IHH and in 1 (5%) healthy woman ($P < 0.001$).

Conclusions

Patients with normal basal serum LH and FSH have it significantly lower oscillations, 2) polymorphisms found in these patients with IHH are not clinically important and 3) disorders of the humoral autoimmunity can be a reason of IHH.

P624

Role of lysosomal acid lipase on the hydrolysis of LDL-transported DHEA-fatty acid esters in HeLa cells

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Background and aims

Dehydroepiandrosterone fatty acid esters (DHEA-FAEs) belong to a unique family of naturally occurring hydrophobic steroid hormone derivatives with long-lasting hormonal activity. DHEA-FAEs function through hydrolysis of DHEA-FAEs into free DHEA and further metabolized to biologically active steroids. The lysosomal acid lipase (LAL) mediates the intracellular hydrolysis of cholesterol esters. This study aims to study the possible role of LAL on the hydrolysis of DHEA-FAE in cultured HeLa cells.

Methods

We used LAL short-interfering RNA (siRNA) oligonucleotides to knock down the expression of LAL in HeLa cells. To control for unspecific RNAi effects, control cells were transfected with nonsilencing siRNA oligos without known similarities to human sequences. We incorporated labelled DHEA-FAEs into LDL by incubating ^{3}H-DHEA with VLDL-free plasma. These siRNA-treated cells were labelled with ^{3}H-DHEA -FAE-LDL for 6 h and chased for 14 and 20 h in the presence of Acyl-CoA:cholesterol acyltransferase inhibitor, PKF 58-035. Cellular and medium fractions were collected and immediately extracted. We used a variety of chromatographic techniques to identify metabolites in the cellular and medium fractions. ^{3}H-cholesterol-oleate-LDL was used as control in parallel experiments to test the validity of the assay.

Results

Anti-LAL western blot showed that most of the LAL protein was depleted by LAL siRNA. After 14 hours chase, more ^{3}H-DHEA-FAE remained unhydrolyzed in the LAL siRNA experiment (71.4%) than that in control experiment (51.8%). When the chase time was extended to 20 h, the difference between two groups disappeared (14.9% and 11.9%, respectively).

Conclusion

Our preliminary studies suggest that LAL is involved in the hydrolysis of ^{3}H-DHEA-FAEs in cultured HeLa cells.

P625

Hydrolysis and metabolism of LDL-transported DHEA-fatty acid esters in HeLa cells

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Background and aims

Dehydroepiandrosterone (DHEA) is an important prohormone and precursor of all sex steroids. DHEA fatty acid esters (DHEA-FAEs) belong to a unique family of naturally occurring hydrophobic steroid hormone derivatives with long-lasting hormonal activity, which are exclusively transported in lipoproteins. In the circulation, DHEA is esterified in a reaction catalyzed by lecithin-cholesterol acyltransferase associated with HDL particles. The physiological role of DHEA-FAEs remains to be clarified. This study aims to study the metabolic fate of lipoprotein-transported DHEA-FAEs in cultured HeLa cells, derived from epithelium of reproductive tract.

Methods

We incubated ^{3}H-DHEA with VLDL-free plasma which resulted in formation of ^{3}H -DHEA -FAEs contained in LDL. We then incubated the labelled LDL particles with cultured HeLa cells in the presence of the ACAT inhibitor, PKF 58-035. After different time points, cells and medium were harvested and immediately extracted with organic solvents. Unesterified ^{3}H-DHEA was separated from ^{3}H-DHEA-FAE by hydrophobic chromatography on Sephadex LH-20, and two-dimensional TLC was used to identify labelled steroid metabolites.

Results

Increased accumulation of ^{3}H-DHEA -FAEs in the cellular fractions were discovered with incubation time up to 24 hours. In the medium fractions, minimal amounts of ^{3}H-DHEA-FAE were observed but increasing amounts of free ^{3}H-DHEA and two metabolites, androstanedione and androstenedione, appeared after 48 hours. Conversely, the cellular fractions only had ^{3}H-DHEA-FAE.

Conclusions

Our preliminary studies suggest that 1.^{3}H-DHEA-FAEs transported by LDL entered HeLa cells via LDL-receptor-mediated intake, 2.^{3}H-DHAE-FAEs were hydrolyzed inside the HeLa cells, 3. The free ^{3}H-DHEA was metabolised into androstanedione and androstenedione, which were secreted into the medium.

P626

Influence of the 5 α-reductase inhibitor type 2 on circulating neuroactive steroids

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The 5α-reductase is one of the enzyme of steroid synthesis and founded in two isoformes. Two distinct 5α-reductase isoenzymes, type I and type II, are found across mammalian species. Each of these isoenzymes is differentially expressed in tissues and during distinct developmental stages and also in different species. The 5α-reductase is enzyme responsible for the reduction of testosterone to dihydrostestosterone, progesterone to dihydroprogesterone and deoxycorticosterone to dihydrodeoxycorticosterone. These steroids and their metabolites (termed neuroactvive steroids) have rapid non-genomic effects on brain function and behavior, primarily via an enhancement of γ-aminobutyric acid (GABA)ergic inhibitory neurotransmission. Neuroactive steroids have through GABA receptor anticonvulsant, antidepressant and anxiolytic effects. Finasteride is the first 5α-reductase type II inhibitor that was introduced to clinical practice in 1992 for the treatment of benign prostatic hyperplasia in the dose of 5 mg/day and few years later for androgentic alopecia in dose of 1 mg/day. There are some reports suggesting finasteride induction of depressive symptoms and anxiety in human. The steroid profile of patients treated by finasteride was detected only in analyse of urine (it was strikingly similar to that of male pseudohermaphrodites with inherited 5 alpha-reductase deficiency). In our study a group of 32 men (12 men with androgenetic alopecia and 20 men wih bening prostatic hyperplasia) was examined. In all individual, their hormonal profile of steroids hormones in blood was determined. Finasteride in the daily dose of 1 mg (men with androgenetic alopecia) or 5 mg (men with benign prostatic hyperplasia) was administrated for 4 months. After the treatment the same hormonal profile was determined. In addition to the decrese of dihydrotestosterone level after treatement, the alteration in other 5 alfa steroids metabolites was found, which could explain the depresive symptomatology.

The study was supported by grant No.NR/8525 – 5 of the IGA MZCR.

P627

Effects of testosterone undecanoate (TU) administered alone or in combination with letrozole or dutasteride in female to male transexuals (FtM)

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TU is an effective option for androgen replacement in FtM subjects, but long-term physiological effects and the significance of T and its metabolites dihydrotestosterone (DHT) and estradiol (E) on physiological functions are unclear. The aim of our study was to investigate the effects of long-term TU treatment on bone metabolism, body composition and lipid profile in FtM subjects and to evaluate the relationship between the observed effects and circulating levels of T, E and DHT. This was a 1-year, randomised treatment, open label, uncontrolled safety study. Ovariectomized FtM were randomly assigned to receive 1000 mg TU injection at week 0, 6, 18, 30, 42 and 54 alone (TU-alone, $n=5$) or in combination with letrozole 2.5 mg, orally (TU + L, $n=5$) or dutasteride 5 mg, orally (TU + D, $n=5$). The Ethics Committee of the S Orsola Hospital approved the study. Outcome parameters included measurement of reproductive hormones, bone metabolism, body composition and lipid profile. Hormones at baseline and at week 54, in group TU-alone, TU + D and TU + L respectively were:
Total T (nmol/l) 7.9 + 6.0 and 13.6 + 2.6; 7.1 + 6.3 and 18.4 + 4.6§; 4.8 + 4.8 and 18.2 + 4.2§; E (pmol/l) 64.4 + 43.4 and 89.6 + 36.3; 41.4 + 19.4 and 76.0 + 47.4; 37.8 + 23.1 and 18.0 + 0.0*; DHT (nmol/l) 1.3 + 1.2 and 2.2 + 0.9; 1.0 + 0.7 and 0.4 + 0.1*; 0.9 + 0.7 and 2.7 + 1.3; *= $P<0.05$% change versus TU-alone; § = $P<0.05$ versus baseline. TU-alone and TU + D treatments were successful in terms of hormone adjustment, did not result in any adverse effects and were well tolerated. BMD decreased by an average of 0.9 g/cm in the TU + L group and the addition of dutasteride resulted in a failure to gain lean mass.
Conclusions
This study confirms that TU is a successful and safe treatment for FtM subjects. Results indicate that E has an important role in bone metabolism and that DHT might play a role in muscle development.

P628

Bilateral cavernous neurotomy induces hypogonadotropic hypogonadism in rat: effect of testosterone and tadalafil supplementation

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A previous study demonstrate that chronic tadalafil administration (2 mg/kg per day) was able to prevent some, but not all, penile alterations induced by long-term (3 months) bilateral cavernous neurotomy (BCN) in the rat. In particular, *in vitro* acetylcholine responsiveness and reduced eNOS and nNOS expression were not preserved, while PDE5 down-regulation, along with muscle/fiber ratio and hypoxygenation (hypoxyprobe) were significantly restored by chronic tadalafil (*J Sex Med* 2006 **3** 419–431). During the course of the previous study a reduction of testis weight from BCN rats was noticed. Aim of this study is to clarify the role of androgens in BCN.
Sprague–Dawley rats were divided in 5 groups: a) control, b) BCN, c) BCN + tadalafil (2 mg/kg per day), d) BCN + testosterone (T, 30 mg/kg per week), e) BCN + tadalafil + T and parameters were recorded as before (*J Sex Med* 2006 **3** 419–431), including hormonal values. Castrated SD rats was used as control.
BCN reduced testis weight, number of Leyding cells, gene expression of the sterodogenetic enzyme 3β-HSD, prostate weight and circulating T, while LH concentration resulted unchanged, suggesting BCN-induced hypogonadotropic hypogonadism. Hypoxygenation, still present in some cavernous endothelial cell in BCN + tadalafil, was completely absent in T-substituted rats. T alone or in combination with tadalafil rescued PDE5 gene level up to control and normalized hyper-sensitivity to the nitric oxide donor SNP. More importantly, T treatment restored eNOS expression and acetylcholine responsiveness. Conversely, nNOS gene expression resulted still down-regulated in T-treated (w or w/o tadalafil) BCN rats.
We described for the first time the presence of hypogonadotropic hypogonadism in long-term BCN, and demonstrated that T substitution can ameliorate the positive effect of chronic tadalafil administration fully restoring penile oxygenation and responsiveness to acetylcholine. The possibility that hypogonadism complicate the radical prostatectomy-associated deleterious effect on penile activity in humans should be tested in forthcoming studies.

P629

Effect of phosphatidylinositol 3-kinase inhibition on estradiol-induced proliferation and hyperplasia formation in the mouse uterus

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It is suggested that phosphatidylinositol 3-kinase pathway is involved in regulation of estrogen effects in the uterus. Therefore, effects of posphatidylinositol 3-kinase blocker, wortmannin, on proliferative and morphogenetic reactions in the uterus under long-term estrogen treatment were examined. Ovariectomized mice were treated with estradiol dipropionate (4 μg per 100 g; s.c., once a week) or vehicle and wortmannin (0.1 mg per 100 g; s.c.; once a day) or vehicle for a month. In animals treated with estradiol and wortmannin, uterine mass was decreased, abnormal uterine glands and atypical endometrial hyperplasia were found rarely. Wortmannin produced a decrease in the numbers of mitotic and bromodeoxyuridine-labelled cells in luminal and glandular epithelia, in stromal and myometrial cells. Levels of estrogen receptors-alpha and progesterone receptors in uterine epithelia, stromal and myometrial cells were increased in mice treated with estradiol and wortmannin. Expression of beta-catenin in luminal and glandular epithelia was also elevated in mice treated with estradiol and wortmannin. Thus, phosphatidylinositol 3-kinase inhibitor wortmannin diminishes proliferative and morphogenetic effects of estradiol. Action of wortmannin is associated with changes in expression of estrogen receptors-alpha, progesterone receptors and beta-catenin in the uterus. This work was supported by grant from RFBR (07-04-00023).

P630

Systemic and seminal antioxidant systems in infertile men with chronic alcohol abuse

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Oxidative stress is associated with chronic alcoholic abuse; it is also a mechanism involved in male infertility. To further investigate the relationships between these two conditions, we studied 6 patients with diagnosis of Alcohol Dependence (DSM-IV) during post-detoxification phase, who consulted our centre for infertility. We evaluated seminal parameters, hormone pattern, Total Antioxidant Capacity (TAC) and the lipophilic antioxidant Coenzyme Q10 (CoQ10), both in blood and seminal plasma. Patients mean age was 40.3 ± 11.8, with history of alcohol consumption of 7 ± 3 years; at the time of the study they were taking low dosages of mood-stabilizers or benzodiazepines. Six age-matched fertile controls were also studied. In a fasting blood sample, testosterone, estradiol, LH, FSH, FT3, FT4, TSH were determined. Standard semen analysis was performed according to WHO guidelines. CoQ10 was assayed by HPLC (in blood plasma it was also normalized for cholesterol levels); TAC was evaluated using the system metmyoglobin-H_2O_2, which interacting with the chromogen ABTS generates a radical spectroscopically revealed; latency time (Lag) in its appearance is proportional to antioxidant content. All patients exhibited as thenozoospemia ($20.5 \pm 2.1\%$ forward progressive motility) and normal hormone values. Blood plasma CoQ10 levels were significantly lower than controls (0.69 ± 0.09 vs 0.81 ± 0.12 μg/ml; CoQ10/cholesterol ratio 164.44 ± 29.10 vs 214.63 ± 17.23 μmol/nmol);similarly Lag was significantly lower (52.5 ± 3.5 vs 76 ± 10 s). On the contrary, a discordance was present in seminal plasma, with CoQ10 lower than controls (0.05 ± 0.01 vs 0.10 ± 0.02 μg/ml), as expected considering asthenozoospermia, but Lag not different from controls (105 ± 27.8 vs 101 ± 5.6 s). These preliminary data confirm a lower antioxidant defence at systemic levels in such patients, but the mechanism of infertility seems to be more complex, not simply related to a local oxidative unbalance.

P631

Development of an Elecsys® Testosterone II Immunoassay with an improved performance for measurement of testosterone in women

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Immunoassays for testosterone produce sometimes incorrectly high results in female samples. The reasons of this phenomenon are not fully understood, but interference by cross-reacting substances and inaccurate calibration can be critical. One known endogenous interfering substance is dehydroepiandrosterone sulphate (DHEA-S). Other substances still have to be identified.
An Elecsys® Testosterone II assay using a new high affinity sheep monoclonal antibody (Bioventix SMA testo3.6A3) is currently in the development pipeline for the Elecsys and cobas e immunoassay platforms. The new assay shows improved recovery if compared with sensitive liquid chromatography tandem mass spectroscopy (LC-MS/MS). In method comparison studies with routine samples versus current Elecsys® Testosterone assay the number of some falsely elevated results is decreased. In addition, the cross-reactivity with DHEA-S and some other potentially interfering substances is reduced. The high affinity of the sheep monoclonal antibody for testosterone enables a low sample volume (20 μl) as well as an inclusion of a high protein content in the assay buffer. As a consequence an enhanced robustness against interferences caused by a variability in the sample matrix is obtained.
The new assay uses a delayed competitive test principle. Firstly, sample is incubated with the biotinylated antibody. Binding sites of the labelled antibody become occupied partly by the sample analyte. Secondly, streptavidin-coated microparticles and ruthenium-labelled hapten are added. Still free binding sites are occupied by the hapten conjugate. Total assay time is 2×9 min. The measuring range of the assay extends from ~0.02 to 15 ng/ml.
The Elecsys® Testosterone II shows improved correlation with LC-MS/MS and provides total imprecision and functional sensitivity that will meet customers needs.

P632

Dynamic changes in the serum inhibin A and B levels correlated to reproductive hormones in women

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To examine the hormonal characteristics related to age of reproductive axis, serum hormone profiles and inhibins A and B were investigated in fertile and menopausal women.
Subjects
Forty-three cycling women (23–40-year-old) and 75 menopausal women (55–70-year-old) were studied. Serum 17-OHP, DHEA, DHEAS, androstendion, estradiol, estrone, progesterone, testosterone, free testosterone, DHT, SHBG, inhibin A and inhibin B, LH, FSH, Prl, TSH, GH, IGF1, insulin, cortisol and thyroid hormones were measured.
Results
Fertile women: The pattern of change in the plasma concentration of inhibin A was: ♦ low in follicular phase negatively correlated to 17-OHP ($P=0.003$), DHEAS ($P=0.008$), progesterone, ($P=0.001$), estrone ($P<0.001$), free ($P=0.04$) and total testosterone ($P<0.001$), dihidrotestosterone ($P<0.001$), total ($P<0.001$) and free triodothyronine ($P<0.001$), and positively with inhibin B ($P=0.023$), TSH ($P=0.003$) and T_4 ($P<0.001$); ♦ significantly increased in mid-cycle, negatively correlated with testosterone ($P=0.032$) and inhibin B ($P=0.021$) and positively with estradiol ($P=0.001$) and hGH ($P=0.025$).
Inhibin B showed a decreasing trend throughout menstrual cycle. In folicular phase, the higher levels of inhibin B were negatively correlated with DHEA ($P=0.002$), androstendion ($P=0.01$), progesterone ($P<0.001$), testosterone ($P<0.001$), free T_3 ($P=0.013$) and hGH ($P<0.001$). In mid-cycle inhibin B negatively correlated with 17OHP ($P=0.04$), estradiol ($P=0.04$), progesterone ($P=0.009$) and positively with testosterone ($P=0.004$).
Menopausal women: Both inhibins significantly decreased. There were no significant correlations between secretion of inhibins and both gonadal and gonadotropin hormones.
Conclusion
The current study suggests that inhibins A and B contribute to follicular maturation by primary mechanism that mediates gonadal steroids action in the reproductive tract.

P633

Polymorphisms of the sex hormone-binding globulin gene contribute to the interindividual variation of gonadal steroid hormone blood levels in men

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Sex hormone binding globulin (SHBG) is a plasma glycoprotein responsible for high-affinity binding and transport of sex steroids. In men there is a large interindividual variation of SHBG levels, and consequently of serum total testosterone (T) and serum total estradiol (E2) levels. Family and twin studies suggested a strong genetic contribution to this variation, besides a hormonal and metabolic influence. The aim of this study was to examine the influence of a missense mutation (Asp327Asn), resulting in an additional N-glycosylation site, and a $(TAAAA)_n$-repeat in the promotor region, resulting in altered transcription, on SHBG and sex steroid serum levels in a population of healthy men covering several decades of adult life. SHBG and hormone levels were measured in 1485 healthy men (aged 24 to 86 years). Carriers of the Asn^{327} allele were identified using restriction analysis; the number of TAAAA-repeats was determined by fragment analysis. Prevalence of the variant Asn-allele was 21.5%. The Asn-allele was associated with higher SHBG (Asp/Asn and Asn/Asn 27.1 nmol/l; Asn/Asn 24.8 nmol/l; $P<0.001$) and T levels (Asp/Asn and Asn/Asn 497 ng/dl; Asn/Asn 471 ng/dl; $P=0.01$), whereas for E2 no differences were found. For the $(TAAAA)_n$-repeat, 7 different alleles were observed ranging from 5 to 11 TAAAA repeats with six, eight or nine repeats occurring the most frequently. Carriers of six TAAAA-repeats presented with significant higher SHBG (13.6%; $P<0.001$), T (9.9%;$P<0.001$) and E2 levels (6.1%; $P=0.002$) compared with non-carriers. For free T a marginal increase was found for carriers, whereas free E2 did not differ between carriers and non-carriers. Our findings show that the Asp327Asn polymorphism and the $(TAAAA)_n$-repeat contribute to the interindividual variation in total serum T levels in healthy men through variation in SHBG concentrations.

P634

Leydig cells activity is impaired in patients with Sertoli cell-only syndrome

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Sertoli cell-only syndrome (SCOS) is characterized by a lack of germ cells and thickened seminiferous tubule walls and is a frequent finding among men with non-obstructive azoospermia. Spermatogenesis is dependent upon the function and number of Sertoli cells (SC) to support the developing germ cells. Klinefelter syndrome is the most frequent genetic cause of SCOS and presents histological features in common with idiopathic SCOS. Aim of this study was to analyze the relationships between histological features and hormonal picture in SCOS patients (n: 37; AZ), Klinefelter patients (n: 14; KL) and vasectomized patients as a control group (n: 14; VA). Testicular tubule wall thickness was different in the three groups ($P<0.05$), with the highest values (12.2 ± 0.9 μm) in KL and the lowest in VA (6.7 ± 1.3 μm). Leydig cells number per grid was different between the three groups, with the highest number in KL (255.7 ± 22.5) and the lowest in VA (48.1 ± 16.4, $P<0.001$). The SC number per tubule was higher in AZ (44.5 ± 2.8) compared with VA (21.8 ± 6.6, $P<0.001$), whereas the difference did not reach significancy between KL (132.0 ± 44.2) and either AZ or VA. Total testosterone levels were higher in VA (15.0 ± 3.7 nmol/l) compared with KL (9.4 ± 1.6 nmol/l, $P<0.02$) and AZ (12.8 ± 0.7 nmol/l, $P<0.05$). A significant negative correlation between the Leydig cell number per grid and total testosterone levels ($P<0.001$) was found analyzing all patients together, but not single groups. SC number per tubule was positively related to total testosterone levels ($P<0.001$) and was negatively correlated with tubule walls thickness in AZ only ($P<0.001$). In conclusion, our data show that lower total testosterone levels are related to a higher number of Leydig cells, thicker tubule walls and a smaller tubule width in all the study groups, thus supporting the hypothesis of a major role of androgens on tubular structure maintenance regardless of genetic or environmental subject characteristics. SCOS patients seem to have lower total testosterone levels as a consequence of an impaired Leydig cells secretive activity.

P635

Effects of Tadalafil on nocturnal penile tumescence and rigidity in normal men: randomized, placebo-controlled, crossover study

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Subjects and methods
We performed nocturnal penile tumescence and rigidity monitoring (NPTRM) in order to study the effects of Tadalafil on sleep-related erections in 23 adult healthy men (age: 34.27 ± 8.48 years). The subjects were randomly administered Tadalafil, 10 mg tablet, the 1st (N1) or the 2nd (N2) night (Group A and B respectively) of 3 consecutive nights of erections' monitoring. In Group A the 3rd night monitoring was regarded as control (N-ctr); in Group B the 1st night. Tadalafil was administered 2 h after last food assumption before bedtime. The NPTRM parameters analyzed were: number of valid erections, total duration of rigidity $\geq60\%$ and $\geq70\%$ maximum rigidity, maximum increase of tumescence and total duration of increase of tumescence ≥30 mm.
Results
Number of valid erections (p N-ctr vs N1: <0.01; p N-ctr vs N2: 0.01), total duration of rigidity $\geq70\%$ (p N-ctr vs N1: 0.03; p N-ctr vs N2: 0.05) and maximum increase of tumescence (p N-ctr vs N1: 0.03; p N-ctr vs N2: 0.03) were significantly higher in nights 1 and 2 after Tadalafil than in control night; no differences occurred between the 2 nights after Tadalafil. Total duration of rigidity $\geq60\%$ (p N-ctr vs N1: 0.03; p N-ctr vs N2: ns) and total duration of increase of tumescence ≥30 mm (p N-ctr vs N1: <0.01; p N-ctr vs N2: 0.06) showed higher values only in night 1 after Tadalafil than in control night. In parenthesis values are mean $\pm$ S.E.M.
Conclusion
Our data suggest that Tadalafil is efficacious in improving sleep-related erections. Furthermore in normal men Tadalafil improved NPTRM also in the second night after 24 h from the drug assumption.

P636

Gonadotropin releasing hormone analogue in cyclophosphamide-induced premature gonadal failure prevention

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Background
Premature ovarian failure (POF) due to cyclophosphamide (Cyc) therapy is well documented in systemic lupus erythematosus (SLE) patients. Studies show that the use of gonadotropin releasing hormone (GnRH) analogues appears to protect women from POF. We present our experience in SLE patients treated with triptorelin.
Methods
Observational study of 15 women diagnosed of SLE who have received concomitant treatment with Cyc and GnRH analogues (triptorelin). Cyc was administered as 6–12 intravenous pulses at a dose of 0.5–1 g/m^2 (adjusted for leucopenia and renal function) every 3–4 weeks, and triptorelin by intramuscular injection at a dose of 0.06 mg/kg every four weeks from the first through the last pulse. All patients were asked about menstrual status (formula, disturbances), gestational formule, reproductive desire. Gonadotropin and estradiol serum levels were determined. We also evaluated the SLICC scale for cumulative SLE damage. The median age at menarchia was 13 years (p25 12, p75 14), SLE duration 7 years (range 2–19). All patients received Cyc due to renal disease and had received corticosteroids (median accumulated dose of 15.4 g). Median SLICC was 1 (range 0–4). They received GnRH analogues (median dose 0.063 mg/kg per m) concomitantly with Cyc (median total dose 16 g; range 7.5–27.7). Median age 24 years (range: 15–35) when beginning Cyc treatment. Median time of follow-up after therapy: 22 months.
Results
All patients had adequate gonadal suppression and 14/15 also had hot flushes as a symptom of hypoestrogenism. Fourteen recuperated menstruation a median of 3 months (range 1–10) after withdrawing GnRH analogues. Although menstrual disturbances increased after therapy, mainly dysmenorrhea, only one patient presents POF, with amenorrhea after 11 months of follow-up, and three patients altered ovarian reserve. There have been no pregnancies after Cyc therapy.
Conclusion
Our data show a low prevalence of amenorrhea and POF, supporting the treatment with triptorelina in these patients.

P637

Topographical effects of functional prostanoid and oxytocin receptors on mid-cycle contractions in isolated human myometrium

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Dysfunctional prostanoid and oxytocin receptors have been implicated in the development of myometrial hyperactivity, causing dysmenorrhoea and infertility. The aim of the present study was to investigate the topographical differences in functional contractile PG and oxytocin receptors in mid-cycle isolated myometrium.

Longitudinal, full-thickness sections of the anterior uterus were obtained from consenting pre-menopausal donors (aged 39–48 years) undergoing hysterectomy for benign disorders. Approval for this study was obtained from the Local Regional Ethics Committees. Specimens were harvested at the follicular stage of the menstrual cycle ($n=14$) and myometrial strips were dissected from lower and fundus ends. For contraction recordings, individual strips were mounted in organ baths under physiological conditions and attached to isometric force transducers. Following tissue equilibration, myogenic responses to vehicle (saline), sulprostone (an $EP_{3/1}$ agonist), $PGF_{2\alpha}$, U46619 (10^{-9} M–10^{-5} M) (a stable thromboxane mimetic) and oxytocin (10^{-12} M–10^{-6} M) were measured. Estimates of maximal effect (E_m) and curve mid-point (pEC_{50}) were expressed as means ± S.E.M. and analysed using two-way ANOVA with Bonferroni's *post hoc* test.

The amplitude of phasic spontaneous activity was 19.6 percent greater in lower segment tissue compared to the fundus ($P<0.001$). Similarly, responsiveness to uterotonins was more pronounced towards the cervix with PG-induced myogenic activity enhanced in a concentration-dependent manner compared to time-matched controls ($P<0.01$–$P<0.001$). In contrast, oxytocin significantly augmented myometrial activity in lower (pEC_{50} 6.99 ± 0.75; $P<0.001$) but not fundus tissues (pEC_{50} 8.59 ± 0.80).

The results indicate that functional regionalisation of the human uterus drives the propagation of intrinsic contractile waves from the lower segment towards the fundus. This may direct retrograde sperm transport and facilitate embryo implantation during the periovulatory stage of the menstrual cycle. Contractile receptor dynamics and mechanotransduction pathways are currently being investigated further to improve the development of treatments for aberrant myometrial function.

P638

The importance of the (TTTA)n polymorphism of aromatase (CYP19) gene in menarche

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Background of study

Twin studies have shown that age at menarche may be subject to hereditary influences but the specific determinants are unknown. Estrogens are known to have an important role in menarche. Since aromatase is responsible enzyme for the conversion of androgens to estrogens, the aromatase (*CYP19*) gene could be a candidate gene for the regulation of menarche. The aim of this study was to investigate the possible association of the *CYP19(TTTA)n* polymorphism with age at menarche.

Methods

We studied 130 healthy adolescent females from a closed community in northwestern Greece. Information on menarche was obtained through interviews. The body mass index (BMI) was recorded. The *CYP19(TTTA)n* polymorphism was genotyped.

Results

The mean age at menarche was 12.9 ± 1.2 years and the BMI = 19.8 ± 2.3 kg/m^2. Genotype analysis revealed 5 *CYP19(TTTA)n* alleles containing 7–11 TTTA repeats. As short allele (S) was characterized the allele with < 9 TTTA and as long allele (L) the allele with ≥9 TTTA repeats. The subjects were subdivided into two groups based on median age of menarche. Group 1 included girls with menarche <13 years and group 2 girls with menarche ≥13 years. Among girls with SS genotypes, 52.9% were in group 1 compared with 47.1% in group 2 and among girl with SL and LL genotypes 45% were in group 1 compared with 55% in group 2. This difference was due to the allele with 7 TTTA repeats, since 65.5% of the girls being homozygous for this allele were in group 1 compared with 43.1% of the girls with other genotypes ($P=0.03$). Furthermore, homozygous girls for the allele with 7 TTTA repeats had earlier menarche (12.45 ± 0.9 years) than girls carrying other genotypes (13.04 ± 1.2 years; $P=0.02$). This difference was independent of BMI.

Conclusion

There is evidence for a genetic contribution of the *CYP19* gene to the age at menarche.

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Weight loss determines a complete remission of the polycystic ovary syndrome in most of obese women

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Obesity is strictly connected with Polycystic Ovary Syndrome (PCOS). However, we still do not know whether a form of PCOS secondary to obesity exists or whether obesity exacerbates manifestations of the syndrome. To answer this question we contacted seventy obese patients with PCOS that attended our Endocrinology Unit in the last 3 years and that lose weight after at least 6 months of dietary treatment. Sixty-five out of 70 patients agreed to take part to the study and gave their informed consent. The diagnosis of PCOS was performed at baseline according to the NIH criteria (oligo/amenorrhea and hyperandrogenism or hyperandrogenemia) and was re-evaluated after weight loss using the same criteria. At baseline 59/65 women executed also a pelvic ultrasonography that confirmed the diagnosis of PCO, whereas only 23/65 repeated it after weight loss. After weight loss PCOS disappeared in 45/65 women (Responders – R), whereas PCOS did not disappeared in 20/65 patients (Non-Responders – NR). This result was not related to the reduction of body weight that did not differ between the two groups (mean reduction of body weight: R −14.3 ± 7.3 kg; NR −14.7 ± 8.8 kg, *P* NS). At baseline NR had higher waist, waist-to-hip ratio (WHR) and androstenedione levels respect to R (waist: R 97.3 ± 10.4 cm; NR 106.5 ± 15.9 cm, $P<0.01$; WHR: R 0.851 ± 0.077; NR 0.915 ± 0.097, $P<0.01$; androstenedione: R 354 ± 147 ng/dl; NR 482 ± 151 ng/dl, $P<0.01$). All the other anthropometric, hormonal, and metabolic parameters did not differ between the two groups. Only R group significantly reduced DHEAS (−0.31 ± 0.89 μU/ml, $P<0.01$), LH (−4.51 ± 8.45 mIU/ml, $P<0.05$), and FSH (−0.99 ± 2.28 mIU/ml, $P<0.05$) levels after weight loss. Insulin resistance and hyperinsulinemia similarly improved in both groups. These data show that a secondary form of PCOS related to obesity does exist and that insulin-resistance and hyperinsulinemia are not the main pathogenetic mechanisms of this variant.

P640

The role of sex hormone-binding globulin (SHBG) and aromatase (CYP19) gene variants in the development of polycystic ovary syndrome (PCOS)

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Background of study

Experimental research supports the hypothesis that fetal exposure to androgen excess may programme *in utero* the development of PCOS in adult life. Potential mechanisms for prenatal androgenization could be a reduced binding capacity of androgens by SHBG or reduced aromatization of androgens by aromatase. The aim of this study was to examine whether the *SHBG(TAAAA)n* polymorphism, known to be associated with SHBG levels and the *CYP19(TTTA)n* polymorphism, known to affect aromatase activity, may play a synergistic role in the development of PCOS.

Methods

We studied 180 women with PCOS and 160 healthy women of reproductive age. The body mass index (BMI) was recorded and the hormonal profile was determined on 3–5th day of menstrual cycle. DNA was extracted from blood leucocytes and the *SHBG(TAAAA)n* and *CYP19(TTTA)n* polymorphisms were genotyped.

Results

Genotype analysis revealed 6 *SHBG(TAAAA)n* alleles with 6–11 repeats and 6 *CYP19(TTTA)n* alleles with 7–12 repeats. Women were subdivided in 4 groups: women with short *SHBG* (≤8TAAAA repeats) and *CYP19* alleles (≤9TTTA repeats), women with short *SHBG*–long *CYP19* alleles, women with long *SHBG*–short *CYP19* alleles and women with long *SHBG* and *CYP19* alleles. Women with PCOS had at greater frequency long *SHBG*–short *CYP19* alleles compared to controls (57.3% vs 42.4%, $P=0.07$). Among patients, those with long *SHBG*–short *CYP19* alleles had the lowest SHBG levels ($P=0.02$), and the highest total testosterone ($P=0.008$), free androgen index ($P=0.002$), DHEAS ($P=0.02$) and testosterone/estradiol ratio ($P=0,03$) compared to other genotypes. This association was independent of age, BMI and insulin resistance indexes.

Conclusion

The *SHBG* and *CYP19* genes may have a synergistic role in the developmental programming of PCOS by affecting androgen bioavailability and aromatization, respectively. Women with long *SHBG(TAAAA)n* and short *CYP19(TTTA)n* alleles may be exposed to excess androgens even during intrauterine life and this may 'programme' the development of PCOS in adult life.

P641

Serum concentrations of atherogenic proteins Neutrophil gelatinase-associated lipocalin and its complex with Matrix Metaloproteinase-9 are significantly lower in women with Polycystic Ovary Syndrome: hint of a protective mechanism?

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Background
Neutrophil gelatinase-associated lipocalin (NGAL) and the Matrix Metaloproteinase-9 (MMP-9) have been considered as important mediators of vascular remodeling and plaque instability. The formation of a complex with NGAL and MMP-9 is crucial for atherotic plaque erosion and thrombus formation. In women with Polycystic Ovary Syndrome (PCOS) the incidence of cardiovascular clinical events is not increased, despite the fact that they display a wide spectrum of risk factors. Since the instability of atherosclerotic plaque is a key factor in the clinical manifestations of cardiovascular disease, molecules challenging the plaque stability should be investigated.
Aim
To determine serum levels of NGAL and MMP-9/NGAL complex in women with PCOS.
Subjects and methods
Forty PCOS subjects were compared with 40 matched for age and BMI controls. In each subject, fasting levels of glucose, insulin, gonadotropins, estradiol, androgens, C-reactive protein (CRP), NGAL and MMP-9/NGAL were determined.
Results
NGAL and MMP-9/NGAL complex levels were significantly lower in PCOS group compared to control one (30.4 ± 24.3 vs 70.7 ± 37.9 μg/l, $P < 0.0001$) and (31.5 ± 26.6 vs 115.1 ± 66.9 μg/l, $P < 0.0001$) respectively. When patients and controls were stratified according to BMI, it was shown that NGAL and MMP-9/NGAL levels were significantly lower in lean ($P < 0.0002$ and $P < 0.0001$ respectively) and overweight ($P < 0.0004$ and $P < 0.002$ respectively) PCOS subjects compared to controls.
Conclusions
These findings indicate that NGAL and MMP-9/NGAL complex, two molecules which activate atherotic plaque erosion, are in lower concentrations in PCOS subjects. The role of NGAL and MMP-9/NGAL complex needs to be further investigated, since suppression of these atheromatous molecules might have a protective role in women with PCOS.

P642

PACAP and PACAP receptors in the testis of cartilaginous fish *Torpedo marmorata*

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The pituitary adenylate cyclase-activating polypeptide (PACAP), isolated for the first time from hypothalamic extracts (Miyata *et al.* 1989), was localized in some peripheral tissues such as adrenal, gastrointestinal tract, ovary and testis (Arimura *et al.* 1991, Watanabe *et al.* 1992, Gathei *et al.* 1993, Shioda *et al.* 1994, Kononen *et al.* 1994). It was previously demonstrated that in the testis PACAP was involved in the steroidogenesis regulation (Lacombe *et al.* 2006). In our investigation we studied the distribution and the expression of PACAP and its receptors, PAC1, VPAC1 and VPAC2, in the testis of a cartilaginous fish, *Torpedo marmorata*, by using immunohistochemistry, *in situ* hybridization, western blot and RT-PCR. We showed that PACAP was highly expressed in *Torpedo* testis as well as PAC1 and VPAC1, while VPAC2 was found to a lesser extent. Particularly, PACAP and its receptors were found in those cells actively involved in the testis steroidogenesis such as Leydig cells (differently from other vertebrates studied so far), pre-spermatogonia and Sertoli cells within cysts containing pre-spermatogonia and spermiated cysts. Further, we showed that in germ cells PACAP and its receptors were expressed during spermioistogenesis. Our data strongly suggest that PACAP has a role in the male reproductive processes of cartilaginous fish *Torpedo marmorata*, intervening in steroidogenesis and in the spermioistogenesis.

P643

Simultaneous profile of prostanoids in pregnant preterm non labouring, term non labouring and term labouring myometrium using ESI-LC-MS

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The aim of this study was to simultaneously profile, using electrospray ionization liquid chromatography mass spectrometry (ESI-LC-MS), prostanoids (PG) produced in samples of: pregnant preterm non labouring (PTNL); term non labouring (TNL): and term labouring (TL) myometrium.
Lower segment samples were obtained at Caesarean section from consenting pregnant women (18–36 years of age) at term (38–41 weeks) and preterm (33 weeks) gestation. Samples were transported to the laboratory and immediately bathed in physiological Kreb's solution ± indometacin (1 μM) for 1 h at 4 °C (samples without indometacin are referred to as untreated (U) or treated (T) if indometacin was present), prior to freezing and subsequent solid phase extraction. Extracts analysed using ESI-LC-MS were quantified using calibration lines made up of commercially available standards. Results are expressed as mean pg/mg protein (as estimated by Lowry method).

PTNL ($n=1$)	TNL ($n=6$)	TL ($n=3$)
In U-PTNL myometrium, the three most abundant PGs (ranked from highest to lowest) were: TXB_2, 6-keto-$PGF_{1\alpha}$ and PGD_2	In TNL(U + T) myometrium, the three most abundant PGs (ranked from highest to lowest) were: 6-keto-$PGF_{1\alpha}$, PGD_2 and $PGF_{2\alpha}$	In TL (U + T) myometrium, the three most abundant PGs (ranked from highest to lowest) were: 6-keto-$PGF_{1\alpha}$, $PGF_{2\alpha}$ and PGD_2

Disruption of the thromboxane/prostacyclin balance may be implicated in the onset of preterm labour. Our data are consistent with the role of prostacyclin as a mediator of uterine quiescence at term and the role of $PGF_{2\alpha}$ as an elicitor of myometrial contractions at term labour.

P644

The incidence of adiponectin gene polymorphism and its relation to serum adiponectin and androgen levels, insulin resistance and clinical parameters in polycystic ovary syndrome

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Aim
The present study was designed to examine the relationship between adiponectin gene (T45G in exon 2) polymorphism and clinical and hormonal characteristics in women with polycystic ovary syndrome (PCOS).
Materials and methods
Ninety-six patients with PCOS and 93 healthy subjects were included in the study. Serum levels of sex steroids, insulin and adiponectin levels were measured. Insulin resistance was evaluated by homeostasis model assessment (HOMA). We used the PCR to examine adiponectin gene T45G polymorphism.
Results
Serum adiponectin levels lower in PCOS patients than control group. There was no statistically significant difference in the incidence of gene polymorphism and genotype distribution between PCOS and control groups. We have shown that polymorphism of T45G adiponectin gene in exon 2 is not related to clinical findings, antropometric parameters, increased serum androgen levels and decreased adiponectin levels in PCOS. Nevertheless, fasting insulin level and insulin resistance were significantly higher in polymorphic group.
Conclusion
Our study suggests that adiponectin gene T45G polymorphism is not related the phenotypic features of PCOS but it plays an important role in the development of insulin resistance in the PCOS.

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Serum osteoprotegerin in polycystic ovary syndrome

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Osteoprotegerin (OPG) is a potent inhibitor of osteoclastic bone resorption. Besides osteoblasts, OPG is expressed by both endothelial and vascular smooth muscle cells. Moreover, elevated serum OPG has been found in conditions associated with insulin resistance such as obesity, diabetes and/or Cushing syndrome.
The aim of the present study was to investigate the relationship between serum OPG and insulin resistance in women with polycystic ovary syndrome (PCOS). In a cohort of 39 women (age 25.3±4.6 years, BMI 25.96±5.34 kg/m^2) PCOS was diagnosed according to Rotterdam criteria. After signing informed consent approved by the local Ethical Committee, blood pressure, steroid hormones, gonadotropins, SHBG, blood glucose, insulin and lipid spectrum were determined in fasting state and homeostasis model assessment for insulin resistance (HOMA-IR) was calculated. In addition, insulin sensitivity was determined as glucose disposal in euglycemic hyperinsulinaemic clamp.
Mean serum levels of OPG were 6.33±1.32 pmol/l. In Spearman analysis, circulating OPG significantly correlated with total cholesterol ($r=0.35$, $P\leq0.03$). In partial correlations, OPG was positively related to age ($r=0.46$, $P\leq0.01$), total cholesterol ($r=0.49$, $P\leq0.01$) and FSH ($r=0.48$, $P\leq0.007$), whereas negative correlations were found between serum OPG and diastolic blood pressure, HOMA-IR and/or HDL cholesterol ($r=-0.5$, $P\leq0.005$, $r=-0.46$, $P\leq0.01$ and $r=-0.45$, $P\leq0.012$ respectively). There was no significant relationship between glucose disposal and serum OPG levels.
In the present cohort of women with PCOS, serum OPG levels were significantly associated with diastolic blood pressure, insulin resistance assessed by HOMA-IR and an adverse lipid profile. A possible contribution of OPG to insulin resistance and vascular endothelial dysfunction in PCOS needs to be further investigated.

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Relation of proatherogenic lipid profile and insulin resistance in PCOS

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Polycystic ovary syndrome (PCOS) is a common reproductive endocrine disorder. The metabolic aspect of disorder is characterized by obesity-related risk factors for cardiovascular disease. The aim of this study was to determine in women with PCOS, indices of lipid metabolism and their oxidation, and to assess possible relation to insulin resistance. We investigated 75 women with PCOS (age: 23.1±5.1 years, body mass index (BMI): 24.9±4.7 kg/m^2) and 56 age and BMI respective controls. In all subjects after an overnight fast, blood samples were collected in follicular phase of the cycle for basal glucose, total-, HDL- and LDL-cholesterol, oxidized LDL (OxLDL), triglycerides, apolipoprotein – A1, B and E, nonesterified fatty acid (NEFA), insulin, testosterone and SHBG. Homeostatic model (HOMA index) and free androgen index were determined. PCOS patients in comparison to controls had increased indices of insulin resistance, basal insulin and HOMA index (14.3±8.7 vs 8.2±3.4 mU/l, $P<0.001$ and 3.2±2.0 vs 1.7±0.7, $P<0.001$ respectively), and deteriorated insulin resistance-related dyslipidemia with decreased HDL-cholesterol (1.2±0.3 vs 1.5±0.3 mmol/l, $P<0.01$), elevated triglycerides (1.0±0.6 vs 0.8±0.4 mmol/l, $P=0.010$), and pronounced LDL oxidation 66.9±33.0 vs 50.2±14.6 ng/ml, $P<0.001$). In conclusion, PCOS women had characteristic dyslipidemia of insulin resistance. Elevated OxLDL, and relation to the insulin insulin ($r=0.389$, $P<0.01$) and HOMA ($r=0.343$, $P<0.01$), is suggestive on premature atherosclerosis in patients with PCOS.

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Nonalcoholic fatty liver disease in women with polycystic ovary syndrome

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Background
Nonalcoholic fatty liver disease (NAFLD) has been associated with insulin resistance. As polycystic ovary syndrome (PCOS) is also associated with insulin resistance, the presence of NAFLD has been investigated in patients with the syndrome and there are data showing an increased prevalence of abnormal aminotransferance activity and/or ultrasonographic evidence of hepatic steatosis.
Objective
In the present prospective study women with PCOS were evaluated with abdominal ultrasonography and biochemical testing in order to assess the presence of NAFLD and to identify factors associated with NAFLD in PCOS.
Patients-methods
Thirty-seven patients (Androgen Excess Society criteria), aged 16–48 years (25.8±7.0), with a BMI 16.8–45.3 kg/m^2 (29.4±7.6) (17 obese, 8 overweight, 12 lean) were studied in the early follicular phase. None of the women had a history of liver disease or reported significant alcohol consumption. None was receiving any medication at the time of the study. They had a clinical examination, a biochemical evaluation (fasting glucose and insulin, lipid profile, liver function tests, FSH, LH, Prl, E_2, testosterone, Δ_4, DHEA-S, SHBG) and underwent a liver and a pelvic ultrasound. Ultrasonography was performed by the same radiologist. Insulin resistance was assessed by homeostasis model assessment (HOMA-IR).
Results
Ultrasonography detected fatty infiltration of the liver in 15/37 patients (41.7%). Patients with abnormal liver ultrasonography were older ($P<0.05$), had a higher BMI ($P<0.01$), waist circumference ($P<0.001$), fasting insulin ($P<0.005$), HOMA-IR ($P<0.005$), serum triglycerides ($P<0.05$), free androgen index ($P<0.05$) and a lower SHBG ($P<0.001$) and HDL-cholesterol ($P<0.005$) versus patients with normal liver ultrasonography. No differences were found concerning levels of aminotransferance activity and the presence of polycystic ovarian morphology on ultrasound. Abnormal aminotransferance activity (SGPT> 40 IU/l) was detected in 8/37 patients (21.6%), 5 of them having abnormal liver ultrasonography. No differences were found between patients with abnormal and those with normal aminotransferance activity.
Conclusion
PCOS patients are at increased risk for developing NAFLD. Despite hepatic steatosis on ultrasound, liver biochemical tests may be normal, so liver ultrasonography is the best option for the detection of NAFLD. Metabolic abnormalities of the syndrome seem to be related with the presence of NAFLD.

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Insulin resistance in patients with polycystic ovary syndrome and idiopathic hirsutism

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Objectives
Hirsutism is a common clinical condition in women during reproductive age and characterized by excessive growth of terminal hair in the androgen-sensitive skin regions. Polycystic ovary syndrome (PCOS) and idiopathic hirsutism (IH) account for most of the cases of hirsutism. Insulin resistance and hyperinsulinemia have been demonstrated in women with PCOS and IH. We intended to investigate the degree of insulin resistance in women with PCOS compared to weight-matched women with IH.
Methods
Thirty women with PCOS (mean age, 31.2±3.7 years; body mass index (BMI), 23.3±2.1 kg/m^2) and 38 women with IH (mean age, 25.0±5.1 years; BMI, 24.9±3.4 kg/m^2) were included in the study. The presence of insulin resistance was investigated by using basal insulin levels, the oral glucose tolerance test, and the homeostasis model assessment (HOMA) score in both groups. Written informed consent was obtained after the procedure had been fully explained.
Results
Patients with PCOS had significantly ($P<0.05$) higher basal insulin levels (26.9±6.1 vs 10.8±6.5 mU/l), HOMA scores (6.3±1.3 vs 2.3±1.7) and FBS (95.4±8.2 vs 82.4±8.9 mg/dl) than patients with IH. Twenty-seven normal-weighted patients (90%) with PCOS and 10 (26.3%) normal-weighted women with IH had HOMA scores of greater than 2.5. Six patients (20%) with PCOS and 2 women with IH (5.3%) had impaired glucose tolerance.
Conclusion
PCOS and IH are associated with insulin resistance independent of total body mass. In women with PCOS, insulin resistance appears more common in both obese and non-obese women compared to women with IH. Hyperinsulinemia appears to play a key pathogenic role in the ovarian androgen overproduction, because of the stimulatory effect of insulin on ovarian steroid production.

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Accumulation of dietary glycotoxins in the reproductive system of normal female rats

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The present study aimed to investigate whether dietary advanced glycation end-products (AGEs) are detected in the ovarian tissue of normal female rats and whether they affect metabolic or hormonal profile.
Normal rats were randomly assigned to regular diet, high (H-AGE) or low (L-AGE) content for 6 months. Age-matched rats fed with regular diet served as controls.
H-AGE rats, demonstrated higher levels of fasting glucose ($P<0.001$), insulin ($P=0.069$), and serum AGEs ($P<0.001$) and testosterone ($P<0.001$), than control and L-AGE rats. In H-AGE rats body weight compared with normal ($P=0.118$) and L-AGE-fed rats ($P=0.35$) did not differ. H-AGE group showed increased AGE localization in the theca interna cells of the ovarian tissue compared to control rats ($P=0.003$). Furthermore, increased receptor for AGE (RAGE) staining was also observed in both granulosa as well as in theca interna cells compared to controls ($P=0.038$ and $P=0.052$ respectively).
These results demonstrate for the first time that administration of high AGE diet in normal female rats is associated with increased deposition of AGEs in the theca cells and of RAGE in the granulosa and theca interna cells of the ovarian tissue compared with the corresponding ovarian compartments of the control and low AGE-fed animals. The metabolic alterations in conjunction with the increased deposition in ovarian tissues of dietary glycotoxins and elevated levels of testosterone in H-AGE-fed animals compared to the controls, suggest an additional impact of environmental factors on ovarian function and these findings need further exploration.

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Reactive nitrogen species in the chemical biology of sperm functions

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Inflammation activates a variety of inflammatory cells, which induce and activate several oxidant-generating enzymes which are able to produce high concentrations of free radicals and oxidants which react with each other to generate other more potent reactive oxygen and nitrogen species such as peroxynitrite ($ONOO^-$) that can damage DNA, RNA, lipids, and proteins by nitration, oxidation, etc., leading to increased mutations and altered functions of enzymes and proteins. Spermatozoa generate small amounts of O_2^- and $NO^{\cdot}$. Under physiological condition these compounds exist at very low concentration; however their local concentrations become significant close the production sites and the formation of peroxynitrite appears likely. Moreover, tyrosine nitration is a widely used marker of peroxynitrite. The nitration of protein residues gives rise to 3-nitrotyrosine which represent a protein modification specific for $ONOO^-$ formation *in vivo*. In the present study we have determined $ONOO^-$ production in semen and its correlation with kinetic features in spermatozoa, and we set out to determine whether protein tyrosine nitration takes place in the same sample. Semen samples from 25 normal fertile donors (control group) and 40 infertile patients affected by idiopathic asthenozoospermia were analysed according to WHO 1999 criteria. After liquification one aliquot of semen diluted to 5×10^6 spermatozoa/ml was stored at -80 °C until determinations. Peroxynitrite concentration was measured through the fluorescence of the DCFDA probe. Protein tyrosine nitration was determined with Western Immuno Blot. Curvilinear velocity and straight line velocity of sperm cells were determined using a Motion Analysis CASA system. The controls exhibited $ONOO^-$ production significantly lower than asthenozoospermic patients (7.32 ± 0.54 vs 27.16 ± 1.58, $P<0.001$); furthermore, $ONOO^-$ exhibited a significant inverse correlation with the motility parameters. Moreover, in the western immuno blot there was an increase in the nitration of the tyrosine residues in the asthenozoospermic samples compared to controls. The present data suggest a critical negative effect of peroxynitrite on sperm motility when spermatozoa concentration is normal. Tyrosine nitration is enhanced by peroxynitrite that affects motility parameters. Thus, a possible pathogenic role in infertile men when asthenozoospermia is the main critical problem may be suggested.

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Apoptosis related signaling in the reproductive system

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Apoptosis is crucial in regulating many aspects of the reproductive system. Since mitogen-activated-protein-kinases (MAPKs) and phosphatidylinositol-3′-kinase (PI3K)-dependent signaling systems play important roles in apoptosis regulation, we undertook to study their involvement in the induction of apoptosis in the reproductive system.
Two pro-apoptotic stimulants were investigated in two corresponding cell lines. One of them was prostaglandin F2α (PGF2α), which is known to be the principal physiologic luteolytic factor in mammals. It was found to exert direct apoptosis in human luteinized granulosa cells, and thereby studied in SVOG-4O cell line. The second stimulant was gonadotropin-releasing-hormone-analog (GnRH-a), which is known to induce direct apoptotis in many malignancies, benign diseases and various cell lines. As a model system we used the mouse pituitary cell line, αT3-1, which was found here to undergo apoptosis upon GnRH-a treatment.
We report that PGF2α and GnRH-a directly induces apoptosis in SVOG-4O and αT3-1 cell lines respectively in a dose and time dependent manner. The apoptotic effect of PGF2α and GnRH-a is mediated by JNK and inhibited by the PI3K-PKB pathway. PKC activation induces the assembly of the catalytic and regulatory subunits of PP2A, thus activating it. Activated PP2A binds to PKB causing its dephosphorylation. Furthermore, PKC activation induces inhibition of PI3K activity. Altogether, the reduction of PKB activity releases PKB-induced inhibition of MLK3, thus further stimulates JNK activity and accelerates PGF2α and GnRH-a apoptotic effect.
We conclude that both PGF2α and GnRH-a exert pro-apoptotic effect via similar signal transduction pathways. Our results support a potential use of GnRH-a for the treatment of various diseases and suggest that the outcome of this treatment can be amplified by using PI3K-PKB inhibitors. Finally, revealing the signal transduction pathways governing apoptosis in luteinized human granulosa cells may help both in the diagnosis and treatment of various reproductive abnormalities, using specific signal transduction modulators.

Signal transduction

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Insulin regulation of proliferation involves activation of akt and erk 1/2 signaling pathways in vascular smooth muscle cells

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This investigation used primary cultured rat vascular smooth muscle cells (VSMCs) to examine the effects of insulin (INS) on proliferation of VSMCs. In this study, protein kinase B (Akt) and p42/44 mitogen-activated protein kinase (ERK 1/2) signaling pathways in mediating the mitogenic actions of INS in VSMCs was investigated. Incubation of a rat VSMCs with INS (100 nM) for 10 min resulted in an increase of Akt phosphorylation by 6-fold ($P<0.001$) and ERK 1/2 phosphorylation by 3-fold ($P<0.001$). Pretreatment for 15 min with 10 μM of P13 K/Akt inhibitor LY294002 or with 20 μM inhibitor of ERK 1/2 PD98059 significantly reduced INS-stimulated Akt and ERK 1/2 phosphorylation by 76% and by 75%, respectively. Incubation of a rat VSMCs with INS resulted in an increase of VSMC proliferation (CONT = 100%, INS = 187 ± 13%, $P<0.001$.) The effect of INS on VSMC proliferation was significantly reduced by 68% by pretreatment with LY294002 ($P>0.01$) and by 71% ($P>0.01$) by pretreatment with PD98059. These results indicate that INS acts through Akt and ERK 1/2 signaling pathways to up-regulate proliferation of VSMC's.

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The hinge region of the human TSH-receptor (hTSHR) mediates the activity of a superagonistic human TSH analog and bovine TSH

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The hinge region of the hTSHR links the extracellular LRR-domain with the transmembrane domain. Recently, we identified mutations E297A, E303A, and D382A in the hinge region with a cell surface expression comparable to the wt but with a strongly reduced bovine TSH (bTSH) binding. The combined triple mutation E297A/E303A/D382A revealed a cell surface expression comparable to the wt but in comparison to the single mutants a more pronounced reduction of bTSH binding (9% of the wt), a decreased maximal cAMP signal of 43% and a 7-fold higher EC50 value than the wt when stimulated with bTSH. To investigate whether the reduced signaling effects of E297A/E303A/D382A are specific for bTSH we determined the triple mutant's cAMP production and EC50 after stimulation with hTSH, which remained at the level of wt hTSHR. The major sequence difference between hTSH and bTSH is the lack of additional positively charged residues in hTSH. To verify potential complementary charge interaction of bTSH and hTSHR we next tested the hTSH analog TR1401 that differs from hTSH by four additional positively charged amino acids, which also exist in bTSH. Indeed, comparable to bTSH after stimulation with TR1401 the cAMP signal of the triple mutation was reduced to 52% of the wt and a 5.6-fold higher EC50 value was determined. Our data indicate for the first time that signal enhancing positively charged amino acids in the hTSH analog TR1401 and in bTSH are likely involved in direct electrostatic interactions with negatively charged residues E297, E303 and D382 located in the TSHR hinge region. This observation implies that the hinge region is a mediator of the superagonistic activity of this hTSH analog and bTSH. Furthermore, the identified intermolecular charged-charged interaction is the first detailed information for the orientation of TSH towards the hinge region of the GPHRs.

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Melatonin acts on pancreatic β-cells via melatonin receptors using the cAMP-, IP3- and probably also the cGMP-signalling pathway

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Physiological and molecular examinations provided evidence for the receptor-mediated influence of the pineal hormone melatonin on insulin secretion of pancreatic β-cells. The predominant effect is a reduction the rate of insulin secretion transmitted by downregulation of intracellular cAMP concentrations. In addition, there is also evidence for an activation of the PLC-IP_3-pathway. Further investigations established the existence of the melatonin-receptor MT1, and recently, also the MT2-receptor subtype could be detected in pancreatic β-cells.
The aim of this study was to examine the involvement of the MT2 melatonin receptor in the insulin-inhibiting effect and whether melatonin may mediate its effect via modulating the cGMP signal transduction pathway. Results of incubation experiments with rat insulinoma cells (INS1) demonstrate that melatonin inhibited the forskolin-stimulated insulin secretion. This effect could be reversed by pre-incubation with the unspecific melatonin receptor antagonist luzindole as well as by the MT2-receptor specific antagonist 4P-PDOT. Measurements of cGMP concentrations using an enzyme immunoassay showed that melatonin significantly inhibited total cGMP levels in a time-dependent manner. This effect could also be reversed by application of luzindole as well as 4P-PDOT, indicating that melatonin modulates cGMP concentrations via the MT2 receptor. Stimulation of INS1 cells with the membrane-permeable 8-Br-cGMP resulted in a dose- and time-dependent increase of insulin secretion. In conclusion, it could be demonstrated that the melatonin receptor subtype MT2 as well as the cGMP signalling pathway are involved in the insulin-inhibiting effect of melatonin. These data improve recent investigations about signal transduction- and receptor-mediated influences of melatonin on the pancreatic β-cells.

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D327N SHBG is more effective than wild-type as modulator of estradiol signalling in breast cancer cells

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Sex hormone-binding globulin (SHBG), the specific plasma carrier for sex steroids, regulates hormone bioavailable fraction and estrogen signalling system in breast cancer cells. A common single nucleotide polymorphism in the human *SHBG* gene results in an amino acid substitution (Asp327Asn), which introduces an additional N-glycosylation site that has been associated with reduced breast cancer risk in postmenopausal women. Since wild-type SHBG was demonstrated to interfere with estradiol signalling, and to inhibit estradiol-mediated proliferation and anti-apoptosis, the present study compares the ability of wild-type and D327N SHBG in modulating estradiol effects in MCF-7 breast cancer cells. Recombinant wild-type and D327N SHBGs were obtained from CHO cells transfected with the two different cDNAs. Recombinant proteins were evaluated as far as their ability to bind estradiol and to interact with cells was concerned; while no difference in estradiol binding was observed, the D327N SHBG bound to MCF-7 cells at a significantly greater extent than wild-type protein. Both proteins were than investigated for their capacity to induce the second messenger cAMP in MCF-7 cell; the levels of cAMP reached after D327N SHBG treatment was again significantly higher that that reached after wild-type SHBG treatment. As well, D327N SHBG was significantly more effective in inhibiting estradiol-induced phosphorylation of Erk 1/2 than wild-type protein. Last, MCF-7 cell proliferation was studied; both SHBGs inhibited estradiol-induced proliferation, but again the D327N form resulted more effective. In conclusion, the polymorphism Asp327Asn of human SHBG confers to this form of protein a protective action in breast cancer, and here for the first time we delineate the mechanism of action, demonstrating that D327N SHBG is more effective than the wild-type protein in modulating estradiol signalling to breast cancer cells.

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Consequences of PRKAR1A (Carney complex gene) inactivation on cellular and subcellular PKA activity monitored by FRET-based reporters

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cAMP/PKA pathway activation is frequently involved in endocrine tumors with overactivity. The Carney complex (CNC) is an autosomal dominant multiple endocrine neoplasia syndrome which associates cardiac myxomas, spotty skin pigmentation and endocrine overactivity. Mutations in the PRKAR1A gene located at 17q22-24 and encoding for the R1A regulatory subunit of protein kinase A have been found in about 60% of CNC. These mutations are heterozygous germline mutations leading to abnormal mRNA generally degraded by NMD (nonsense mediated decay) and thus no abnormal protein is expressed. Loss of heterozygosity (LOH) at 17q22-24 can be observed in tumors, suggesting that PRKAR1A is a tumor suppressor gene.
The aim of this study is to investigate the consequences of *PRKAR1A* mutations on PKA activity at the cellular and subcellular levels. We use the silencing RNA method to inactivate R1A and obtain at least a 80% decrease in PRKAR1A protein content in HEK293 cells. PKA activity is monitored by fluorescent resonance energy transfer (FRET) using A-kinase reporters (AKARs) in the whole cell (AKAR3) and in different subcellular compartments (cytosol: ELS-AKAR3, nucleus: NLS-AKAR3, mitochondria: dAKAP-AKAR3, plasma membrane: PM-AKAR3). The FRET ratio is measured in basal conditions and after cAMP pathway activation with forskolin or prostaglandin E1 (PGE1). R1A inactivation is associated with a two-fold up to four-fold increase of basal and

stimulated PKA activity in whole cells compared with control. Targeted fluorescent probes show different subcellular patterns of PKA activity alterations after R1A inactivation.These results demonstrate at the cellular level that PRKAR1A mutations augment PKA activity and suggest that this effect differ between subcellular compartments.

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Orexins stimulate steroidogenic acute regulatory (StAR) protein expression through multiple signalling pathways

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Orexins mediate a variety of physiological processes including feeding behaviour, the circadian pathway and cortisol secretion. Steroidogenesis is regulated by a variety of neuropeptides and one of the key rate-limiting steps is cholesterol transport across the mitochondrial membrane by the steroidogenic acute regulatory (StAR) protein. StAR expression can be regulated through several different signalling pathways. Despite the clear link between orexins and steroid production, the actions of the orexin family of hormones on steroid biosynthesis are not fully understood. We present data showing that 100 nM both ORA and ORB for 4 or 24 h significantly up- StAR, in H295R pluripotent adrenocortical cells. We further assessed the dose-dependent and time-dependent characteristics of StAR up-regulation at the protein level, showing significant increases after 4 h at a relatively low agonist concentration (1 nM). We have provided a key analysis of the precise G-protein coupled signalling pathways required for the up-regulation of StAR protein in response to ORA and ORB. This has involved dominant negative G-protein analysis and the direct inhibition of the PKA, PKC, ERK1/2 and p38 pathways. This shows a fundamental role for multiple G-protein coupled and MAPK-mediated signalling pathways leading to StAR expression. Antagonist analysis also showed that orexin effects on StAR were primarily (but not exclusively) acting through the OX1R. This is the first study linking orexin action on StAR expression and comprehensively describes the signalling pathways involved in regulating the complexity of hormone biosynthesis.

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G protein coupling and adenylyl cyclase inhibition are mediated by the DRY motif in the second intracellular domain of the SST5 receptor

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Somatostatin exerts inhibitory effects on hormone secretion and cell proliferation by interacting with five different receptors (SST1–SST5) linked to effector systems via G proteins. The receptor structural domains mediating these effects have not been identified. Since SS is the hypothalamic peptide that physiologically inhibits GH secretion, aim of this study was to investigate the molecular determinants mediating the interaction of the human SST5 with G proteins and subsequent signal transduction after stimulation with BIM23206, the SST agonist with highest affinity for SST5, in the rat pituitary cell line GH3. The most critical regions important for signal transduction in different GPCRs are the second and the third intracellular loops (i2 and i3). To investigate the role of these regions in SST5 receptor, we focused on the highly conserved DRY motif (Asp-Arg-Tyr) in the i2 loop and the BBXXB domain in the i3 loop. We introduced mutations into SST5 replacing D^{136} and R^{137} residues in the DRY motif with Ala, and we used a naturally occurring mutant (R240W) previously found in one acromegalic patient resistant to somatostatin analogues in which the BBXXB domain is altered. By analyzing adenylyl cyclase inhibition mediated by BIM23206 in cells transfected with wt or mutated SST5, we have found that residues D^{136} and R^{137} are important for adenylyl cyclase inhibition (maximum 15% inhibition versus 53.4% of wt), whereas substitution of basic residue R^{240} resulted in mutant receptor that normally activates effector (maximum 50.5% inhibition), but shows a persistent inhibition of cAMP after agonist pretreatment. Our data suggest that an intact BBXXB domain is not crucial in mediating G protein activation but it is involved in desensitization processes, whereas the DRY motif within the i2 loop is crucial in G protein interaction and adenylyl cyclase inhibition, thus suggesting a specific role of i2 loop in SST5 function.

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Ligand-dependent internalization of somatostatin receptors

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Somatostatin (somatotrophin release inhibiting factor; SRIF) is produced primarily in the hypothalamus and pancreas and inhibits the secretion of many hormones and neurotransmitters. Moreover, SRIF and SRIF analogs are able to inhibit tumor growth. Many human neuroendocrine and non-neuroendocrine tumors express the five known somatostatin receptors (sst_{1-5}) in different amounts.
Tachyphylaxis after prolonged treatment with octreotide (Sandostatin) is a well known clinical problem in patients with GEP-NET tumors. Pasireotide (SOM230), which activates $sst_{1,2,3}$, and $_5$, in contrast to octreotide which activates sst_2 primarily, showed only little tachyphylaxis of hormone secretion in preclinical assays. In order to understand the mechanism underlying receptor activation and tachyphylaxis, we studied somatostatin receptor subtype trafficking and function.
In HEK293 cells stably expressing recombinant human sst_2, we found that octreotide, but not pasireotide (1 μM), caused a rapid (within 5 min) and complete internalization of these receptors from the cell membrane to the cytoplasm. Complete recycling of the receptors to the cell membrane was observed after 5 h. Interestingly, both compounds were similarly effective in inhibiting forskolin-induced cAMP production (up to 60% inhibition after 15 min at a 1 μM dose) in these cells. Subsequently we extended our study to the human hepatocellular carcinoma cell line HuH7. Using immunohistochemistry on these cells we detected $sst_{3,4}$ and $_5$, but no sst_1 and sst_2. Pasireotide was able to significantly inhibit cAMP production (40% inhibition at 1 μM), whereas octreotide was not effective. Preliminary confocal studies showed that pasireotide had no significant effect on sst_3 or sst_5 localization in HuH7 cells.
These data provide the first information on the specific effects that octreotide and pasireotide exert on the different sst subtypes and give insight in the molecular properties of these two compounds concerning the induction of tachyphylaxis.

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Combined rapamycin-octreotide treatment exerts its superior anti-proliferative action by dramatically increasing p27/Kip1 expression

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Combined rapamycin-octreotide treatment has a stronger antiproliferative effect on non-functioning pituitary tumor cells compared with each drug alone. The mechanism of the combined treatment action was studied in AtT-20 pituitary tumor cells. Rapamycin is known to induce cell cycle arrest at the G1/S transition point. The entry of eukaryotic cells into the cell cycle is controlled primarily by the activation of cyclin-dependent kinases (CDK4/CDK6 and CDK2) by their respective cyclin partners (cyclin D1, D2, D3, and E). D-type cyclins form catalytically active kinase complexes with CDK4 and CDK6 which phosphorylate and inactivate the tumour suppressor Rb. Cyclin E forms complexes with CDK2 completing the phosphorylation and inactivation of Rb and leading to irreversible entry into the S-phase of the cell cycle. Cyclin D/CDK4 and cyclin D/CDK6 activity is inhibited by members of the INK4 CDK inhibitor family (e.g. p16/INK4A), while cyclin E/CDK2 is inhibited by p21/Cip1 and p27/Kip1. Rapamycin treatment decreased cyclin D1/D3 and CDK4/6 levels, while octreotide had no effect. Addition of octreotide to rapamycin did not potentiate rapamycin's effect. In contrast, both rapamycin and octreotide increased p27/Kip1 and decreased Cdk2 and cyclin E levels and their combination The two drugs together potentiated their action, providing a mechanism for the better antiproliferative effect of the combined compared to the single treatment. It is important to note that p27/kip1 is a tumour suppressor gene that plays a significant role in pituitary tumorigenesis, since p27 knock-out mice present with pituitary tumours and p27/Kip1 protein is dysregulated in many human pituitary adenoma types. Therefore, the superior effect of the combined treatment, compared to each drug alone, on p27/Kip1 expression may in part explain its better antiproliferative action in pituitary tumor cells.

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NOD proteins expression and function in the pituitary folliculostellate cells

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Folliculostellate (FS) cells were found to regulate hormonal secretion in pituitary endocrine cells. This cell type share properties with components of the innate immune response such as dendritic cells and macrophages, and it is suggested that they are able to initiate immune responses and act as part of an immunoendocrine regulation system. Members of toll-like receptor (TLR) family recognize bacterial cell wall components and initiate an immune response. Recently, we detected by RT-PCR the TLR4 mRNA expression in FS cell line, TtT/GF. In the present study, we detected the constitutive expression of two members of a novel nucleotide-binding oligomerization domain (NOD) family of proteins that act as cytosolic receptors. We show that NOD1 and NOD2 are expressed in the human and mouse pituitary glands, and in TtT/GF cells. Furthermore, NOD1-agonistic diaminopimelic acid (DAP) and NOD2-agonistic muramyl dipeptide (MDP) induced (NF)-κB activation and ERK1/2 phosphorylation in TtT/GF cell cultures. Both NOD agonists in combination with TLR4 agonistic, LPS enhanced NF-κB transcriptional activity, and interleukin-6 (IL-6) production in these cells. Knocking-down NOD1 and NOD2 markedly inhibited LPS induced IL-6 production in TtT/GF cells. RNA interference for NOD2, but not NOD1, downregulated NF-κB activity. LPS, MDP and LPS plus MDP increased NF-κB expression and transcriptional activity, in a mechanism involving STAT3, since knocking-down this protein abolished NF-κB activation. All together, we revealed that NOD1 and NOD2 expression expressed in TtT/GF where they mediate LPS signals in these cells. Finally, these findings suggest that FS cells play a predominant role in the context of immune-endocrine interactions during bacterial infection and inflammation and NOD proteins have a central participation in these processes.

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Corticotropin releasing hormone receptor signalling pathways and regulation of NOSIII activity in syncytialized BeWo cells

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Placental corticotrophin releasing hormone (CRH) appears to play important physiological roles during pregnancy and labour. In the placenta, CRH appears to regulate diverse actions such as human trophoblast cell grown and invasion, tissue remodeling, direct modulation of the placental prostaglandin generation and bioavailability and control of placental vascular tone through regulation of endothelial NOS (NOSIII) expression and activation[1]. CRH actions are mediated through expression of both R1 and R2 CRH receptors (CRH-R) in human syncytiotrophoblasts. To characterize the signaling properties of syncytiotrophoblast CRH-R and identify the mechanisms regulating CRH-NOSIII interactions, we used the cytotrophoblast cell line (BeWo derived from choriocarcinoma) which responds to increased intracellular cAMP levels by differentiation into a multinucleated 'cell' with a syncytiotrophoblast phenotype[2]. Results showed that syncytialization of BeWo cells by forskolin treatment for 24 h, increased by 3× CRH-R1 mRNA expression. Indirect confocal analysis identified strong CRH-R expression primarily around the plasma membrane and diffuse, low level specific staining throughout the cytoplasm. Analysis of the functional characteristics of CRH-R demonstrated that activation of CRH-R by CRH led to significant activation of ERK1/2 and p38MAPK through pathways involving EGF-R transactivation but not PI3-K activation. CRH-induced ERK1/2 and p38MAPK activation was transient (maximal response at 5 min) and confocal microscopy studies showed that activated ERK1/2 was primarily but not exclusively localized to the cytoplasm. Interestingly, cAMP studies suggested that the BeWo CRH-Rs are not coupled to the adenylyl cyclase/cAMP pathway. Moreover, CRH appears to activate the PKB/ Akt and this leads to downstream phosphorylation of NOSIII at serine 1177, a signaling event associated with NOSIII activation. We conclude that syncytialized BeWo cells express functional CRH-R that can regulate distinct signaling cascades generating messengers potentially important for placental biology.

1. Hillhouse EW & Grammatopoulos DK. *Reproduction* 2002 **124** 323–329.
2. Wice B, *et al*. *Exp Cell Res* 1990 **186** 306–316.

P663

Modulation of endothelial NF-κB transcription factors by carbohydrates

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Objectives
It is well known that compounds of follicular fluid, which is rich in carbohydrates, are able to regulate the expression of angiogenesis-related genes in endothelial cells. Nuclear factor κ B (NF-κB) transcription factors are important candidates in modulating the expression of these genes. The aim of our research is to determine if NF-κB factors are maybe involved in the regulation of endothelial angiogenesis-related genes and thus in ovarian angiogenesis.
Methods
Pooled human endothelial cells were incubated with various carbohydrates in such concentrations, which were found in follicular fluid of healthy women. Control cells were cultivated without supplementation. After incubation for four days, endothelial cells were lysed and nuclear as well as cytoplasmic proteins were isolated. Afterwards, the amount of NF-κB factors (cRel, RelB, p50, p52, p65) was quantified by ELISA and compared between stimulated and control cells.
Results
The factors p50 and p65 predominated in the nucleus of unstimulated cells whereas cRel and RelB were found almost exclusive in the cytoplasm. Factor p52, however, was distributed equally in both cell compartments. In the nucleus of stimulated cells, p52 and p65 showed significant increases of 67% and 44% when compared to controls. In the cytoplasm, p52 and p65 were increased significantly at 52% and 65% in treated endothelial cells versus untreated controls. The factors cRel, RelB, and p50 showed no reaction to carbohydrate treatment at all.
Conclusion
Our results indicate that normal carbohydrate concentrations also are able to regulate NF-κB transcription factors suggesting that these substances may play a role in ovarian angiogenesis.

P664

cAMP/PKA and ERK1/2 dependent feedback mechanisms regulating type 2 CRH receptor signalling

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Corticotropin-releasing hormone (CRH) and its related peptides, the urocortins (UCNs) mediate their effects by binding to two types of GPCRs, CRH-R1 and CRH-R2. In most target tissues, the adenylyl cyclase/cAMP/PKA and the mitogen activated protein kinase (MAPK) are the two main pathways mediating the biological effects of CRH-Rs. For most multi-signal G-protein coupled receptors (GPCRs), there is considerable level of 'cross-talk' between different signalling cascades, which is important for determining the overall biological response. In the present study we investigated potential signalling interactions between the cAMP and MAPK cascades following stimulation of HEK293 cells overexpressing recombinant CRH-R2β with UCN-II. Using specific kinase inhibitors we found that CRH-R2β mediated ERK1/2, but not p38MAPK activation was PI3-K dependent, and optimal ERK1/2, but not p38MAPK activation was dependent on an intact cAMP/PKA/AKAP pathway. Furthermore, inhibition of the ERK1/2 pathway by pre-treatment with the specific MEK1/2 inhibitor (UO126) attenuated UCN-II-induced ERK1/2 activation and cAMP responses. These observations may be directly related to CRH-R2β trafficking and endocytosis. Results from Indirect-confocal microscopy studies suggest that inhibition of either PKA (by myr-PKAi) or the ERK1/2 pathway accelerates CRH-R2β endocytosis, which was additionally confirmed by loss of CRH-R2 from the cell membrane. Indirect confocal analysis and immunoblotting using phospho-specific antibodies determined that UCN-II-activated ERK1/2 appeared to target β-arrestin1 (βarr1) and modulate, through phosphorylation at Ser412, its translocation to the plasma membrane, binary complex formation with CRH-R2β and receptor internalization kinetics. Depletion of endogenous βarr1 by siRNA reduced CRH-R2β internalization and enhanced UCN-II- induced ERK1/2 and p38 MAPK activation by 80% and 55% respectively. These findings reveal a complex interplay between the cAMP-ERK1/2 pathways that might allow 'fine-tuning' of CRH-R2β functional responses.

P665

Signalling properties of corticotropin-releasing hormone receptor (CRH-R1/2) in adrenal (H295R) cells

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Corticotropin-releasing hormone (CRH) and urocortins (UCN) bind to two types of GPCRs (CRH-R1 and CRH-R2), activating a plethora of signalling cascades, including the mitogen-activated protein kinases (MAPKs) pathways. Although previous studies have identified that CRH directly regulates adrenal steroidogenesis[1], the second messenger systems and downstream effectors remain poorly defined. The adrenal cell line H295R, which express both CRH-Rs[1] was used to investigate CRH-R signalling characteristics and UCN-I effects on enzymes involved in adrenal steroidogenesis. Confocal analysis identified receptor 'hotspots' on the plasma membrane and diffuse, low level specific staining throughout the cytoplasm, which may represent unprocessed receptors or cytoplasmic CRH-R variants. Functional coupling of the activated receptors to Gs/adenylyl cyclase resulted in a 3–4 fold increase in cAMP levels following treatment of cells with 100 nM UCN-I. Using phospho-specific antibodies for ERK1/2 we observed a transient increase in ERK1/2 activation (maximal response 4–7 fold above basal at 5–10 min) in response to UCN-I (100 nM). However, UCN-I did not induce any significant activation of p38 MAPK. Confocal microscopy studies showed that active ERK1/2 was exclusively localised to the cytoplasm at the time points measured (0–30 min). UCN-I treatment for 4 h significantly induced StAR mRNA and protein levels, and appeared to involve ERK1/2 activation since pretreatment with the specific MEK1/2 inhibitor (U0126) abolished this effect. Real-time PCR experiments also showed that treatment of H295R cells with 100 nM UCN-I for 24 h significantly up-regulated CYP11A, CYP17 and 3βHSD. However, ERK1/2 activation was found to be important for CYP17 and 3(HSD but not CYP11A up-regulation. We conclude that H295R cells express functional CRH-Rs, which couple to both cAMP and ERK1/2 signalling pathways. UCN-I can induce expression of specific enzymes involved in adrenal steroidogenesis through an ERK1/2-dependent pathway.

(1) Willenberg HS *et al*., *Neuroendocrinology* 2005 (82) 274–281.

Steroid receptors

P666

Thyroid receptors play a suppressor role by reducing pituitary tumor transforming gene 1 in human hepatocellular carcinoma cells

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Pituitary tumor transforming gene 1 (PTTG1) is expressed in most tumors. However, whether thyroid hormone (T_3) and its receptors (TRs) regulate PTTG1 in human hepatocellular carcinomas (HCC) remains unclear. Previous cDNA microarrays revealed PTTG1 is down-regulated by T_3/TR. This study investigated the significance of PTTG1 regulation by T_3 in HCC cells. The PTTG1 mRNA and protein expression were repressed by T_3 in HepG2-TR cells over-expressing TR. Similar results were observed in thyroidectomized rats. To localize the regulatory region in the *PTTG1* promoter, serial deletions of mutant *PTTG1* promoters were constructed. The promoter activity of the *PTTG1* gene was repressed (25–51%) by T_3. Additionally, these findings indicate that PTTG1 may be regulated by Sp1 transcription factor. The critical role of -594 and -520 Sp1 binding sites was confirmed by electrophoretic mobility shift assay (EMSA). Transfection with Sp1 expression vector enhanced *PTTG1* promoter fragment reporter activity. Also, Sp1 was down-regulated in HepG2-TRα1 cells and in thyroidectomized rat models following T_3 treatment. Additionally, ectopic expression of PTTG1 promotes cell proliferation in Hep3B hepatoma cells. Conversely, knock-down of PTTG1 or Sp1 expression reduces cell proliferation in HepG2 cells. Also, TR expression was reduced in HCC over-expressing PTTG1. Together, these findings indicate that *PTTG1* gene expression is mediated by Sp1 and is indirectly down-regulated by T_3. Finally, over-expression of PTTG1 or SP1 in HCCs is TR-dependant and crucial in the development of HCC.

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Modulation of creatine kinase specific activity by triiodothyronine in rat organs

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3,5,3′-triiodothyronine (T3), regulates energy metabolism by oxygen consumption and thermogenesis via induction of NaK-ATPase *in vivo*. These changes are correlated with nuclear T3 binding in responsive organs. We showed that creatine kinase (CK) another ATP regulating enzyme, is modulated by different hormones, in tissues containing appropriate receptors. This modulation is via binding to specific receptors and changes in CK mRNA levels. Since CK is involved in energy metabolism, we examined T3 effects on CK activity in tissues containing T3 receptors, such as kidney (Ki), cerebrum (cx), cerebellum (cbl), epiphyseal cartilage (Ep) and diaphyseal bone (Di). In hypothyroid rats, basal CK activity increased in different tissues and the normal developmental pattern of CK was partially maintained. T3 injection into hypothyroid rats lowered CK activity in Ki, cx and Di but increased it in cbl and Ep. Hormonal injection into euthyroid rats resulted in increased CK only in cbl and Ep. Parallel changes were detected in CK mRNA expression and stimulation of DNA synthesis in the different organs. Adenylate kinase, another energy metabolising enzyme did not change by thyroid status or treatment. These results suggest that T3 modulates CK activity both directly and indirectly via changes in ATP levels. In different organs except cbl and Ep, changes in CK may be compensatory to changes in NaK-ATPase. In Ep and cbl where T3 increases cell proliferation, it may also induce CK directly since we have shown its correlation with stimulation of cell proliferation.

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Testosterone increases kidney weight in orchiectomized male Wistar rats but not dihydrotestosterone

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Introduction
Androgens are known to play an important role in renal tubular epithelial cell growth, hypertrophy and erythropoetin production, however the exact mechanisms are not clear yet. 5alpha-dihydrotestosterone is synthesized primarily in gonads and skin, and is the most used androgen in studies. However, little is known about testosterone effects in non-gonadal tissues.
Methods
Male Wistar rats aged 8–10 weeks were orchiectomized and put on a low- or high-salt diet over 5 weeks. In addition they received either placebo, testosterone (1 mg/animal) or 5alpha-dihydrotestosterone (DHT) (1 mg/animal) as daily s.c. injection over 16 days (each group $n=6$). In additional experiments, rats were treated with mineralocorticoid antagonist spironolactone (50 mg/kg weight per day), which excerts anti-androgenic properties, or androgen receptor antagonist flutamide (30 mg/kg weight per day). Blood and organs were secured after decapitation.
Results
Prostate weight was reduced in ovariectomized rats (20 ± 5 mg); testosterone and DHT treatment significantly increased prostate weight (377 ± 35 and 103 ± 37 mg respectively). Flutamide treatment completely abolished this effect; spironolactone did not show an effect. Testosterone serum concentrations reached 9–13 ng/ml in testosterone and 0.6–1 ng/ml in DHT treated rats; testosterone levels were higher in flutamide than in spironolactone treated animals. DHT serum concentrations were 0.8–1.6 ng/ml in testosterone and 0.5–1.3 ng/ml in DHT treated rats. No significant differences were observed in estradiol concentrations between the groups. Absolute kidney weight increased significantly in testosterone but not in DHT treated animals. The testosterone effect was reversed by flutamide, but not by spironolactone treatment. In addition, body weight increase was higher in testosterone treated than in control animals, and the effect was significantly diminished by flutamide and by spironolactone treatment.
Conclusions
These results highlight the anabolic effects of testosterone in non-gonadal tissues, especially in kidney epithelial cell growth and hypertrophy. These results indicate the need for further studies of testosterone effects.

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_Xba_I polymorphism of ESR1 gene might influence the effect of raloxifene on the endothelial function

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Objectives
The selective estrogen receptor modulator, raloxifene, exerts a part of its actions through the estrogen receptor alpha activation. We explored if polymorphisms of ESR1 gene modify the effects of 6 months raloxifene treatment on endothelial function.
Methods
Two intronic (*Pvu*II and *Xba*I), and one exonic polymorphism (*P325P*) of ESR1 were analyzed in 53 postmenopausal women, mean age 59.7+6.2. The flow mediated endothelium dependent vasodilation (FMD) and cell adhesion molecules (CAM) ICAM-1, VCAM-1 and E-selectin, were measured before and after 6 months of raloxifene treatment. SSCP method was used to determine the *P325P* and RFLP to determine *Xba*I and *Pvu*II polymorphisms.
Results
There was no relation between ESR1 genotypes and either FMD or CAM levels, at baseline. After 6 months of raloxifene treatment, the FMD was significantly greater in subjects with XX genotype of *Xba*I polymorphism compared to xx, and of borderline significance compared to Xx genotype ($P=0.03$ and $P=0.053$, respectively). Neither the *Pvu*II nor *P325P* ESR1 gene polymorphisms influenced the FMD after 6 months of treatment. None of the ESR1 gene polymorphisms influenced the levels of CAM. When analysing the whole group, a significant decrease in E-selectin and a significant increase in ICAM-1 levels, independently of genotypes was observed ($P<0.001$ and $P=0.029$, respectively), but no influence on VCAM-1 level and FMD, was found.
Conclusion
Our data suggest that *Xba*I polymorphism of ESR1 gene might influence the beneficial effect of raloxifene treatment on endothelial function. This effect could be of significant farmacogenomic and clinical importance.

P670

Altered androgen metabolism and signalling during tissue remodelling of the prostatic stroma: implications for prostate cancer and benign prostatic hyperplasia

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The human prostate is highly susceptible to benign and malignant proliferative changes and is associated with the development of benign prostatic hyperplasia (BPH) and prostate cancer (PCa), two of the most common diseases affecting elderly males. The diseased-associated stroma undergoes significant remodelling, including fibroblast-to-myofibroblast/smooth muscle cell transdifferentiation. Subsequent increased secretion of cytokines and growth factors generates a growth favouring microenvironment that promotes stromal hyperplasia and neoplastic transformation of pre-malignant epithelial cells. Microarray and quantitative PCR of *in vitro* transdifferentiated primary prostatic stromal cells changes in enzymes that regulate local androgen metabolism and signal transduction. We demonstrate that prostate-associated gene 4 (PAGE4), a cancer/testis antigen, is a novel corepressor of the androgen receptor (AR). Overexpression of PAGE4 inhibits mRNA and protein expression of endogenous androgen-regulated genes and also inhibits AR-mediated transactivation of hormone-responsive reporter constructs. Yeast two hybrids screening indicate that PAGE4 may repress AR transactivation by sequestering AR coactivator proteins, such as nuclear receptor interacting protein (NRIP). PAGE4 is induced during fibroblast-to-myofibroblast transdifferentiation and is up-regulated in BPH stroma indicating that repression of the androgen signal by PAGE4 may facilitate stromal tissue remodelling and thus disease development/progression. PCa progression to hormone refractory status is associated with aberrations in AR signalling that permit continued AR transactivation despite androgen withdrawal treatment. PAGE4 expression is downregulated during PCa progression and its absence may provide a mechanism by which AR activity is maintained in advanced disease. Collectively, these data indicate that therapies designed to target PAGE4 may be of clinical benefit in BPH and PCa.

P671

(Lack of) Epigenetic variability in the human glucocorticoid receptor promoter CpG island

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Tissue and cell type specific control of glucocorticoid receptor (GR) levels is thought to occur through the usage of alternate promoters, and associated non-coding alternate first exons. The vast majority of the known alternative first exons are located within a 3.2 kbp CpG island. Methylation of certain CpG dinucleotides within the rat *Gr* promoter has been implicated in changes in hippocampal *GR* expression levels and the resultant disturbances in the HPA axis. Bisulphite sequencing of rat hippocampal samples has previously shown that the two CpG di-nucleotides within an NGFI-A binding within the rat exon 1_7 promoter (human homologue 1F) can be methylated, and that environmental changes (handling, or maternal care) induce epigenetic programming via differential methylation of the 5′ CpG di-nucleotide. Methylation of this di-nucleotide reduces NGFI-A induced promoter activity.
The available data suggests that in post mortem human brain samples there is no visible methylation of the corresponding NGFI-A CpG di-nucleotides. For both the rat and the human, the available data covers 100 bp of promoter 1_7/1F; however, there is no data available as to methylation patterns elsewhere within the CpG island where the majority of these alternate first exons are found.
Using readily available PBMC we performed bisulphite sequencing on promoters 1D, E, F, and H, covering approximately 45% of the complete CpG island for 26 healthy subjects (22 female and 4 male, age range 35–67, mean 51±8 years). Analysis of our data suggests that, as previously reported for human hippocampal post morten samples, the NGFI-A binding site is not methylated, and that within the context of the larger CpG island, methylation of the human GR promoter is rare. Our data suggests that epigenetic mechanisms do not play a role control of human *GR* transcription.

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The vitamin D receptor agonist elocalcitol up-regulates L-type Ca^{2+} channel activity in human and rat bladder

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Human bladder contraction mainly depends on Ca^{2+} influx, via L-type voltage-gated Ca^{2+} channels, and on RhoA/Rho-kinase contractile signalling, a pathway up-regulated in overactive bladder (OAB) syndrome. Elocalcitol (Elo) is a vitamin D receptor agonist inhibiting RhoA/Rho-kinase signalling in rat and human bladder, shown to ameliorate OAB symptoms in a clinical study. Since in normal bladder from Sprague–Dawley (SD) rats Elo treatment delayed the carbachol-induced contraction without changing maximal effect, we hypothesized, based on increased sensitivity to the selective L-type Ca^{2+} channel antagonist isradipine in bladder strips from Elo-treated rats, an up-regulation of L-type Ca^{2+} channels. Thus, this hypothesis was further investigated. In human bladder smooth muscle cells (hBCs), Elo induced a rapid increase in intracellular [Ca^{2+}], which was abrogated by the specific L-type Ca^{2+} channel antagonist verapamil and was undetectable in the absence of extracellular Ca^{2+}. Moreover, hBCs exhibited voltage-activated Ca^{2+} currents (I_{Ca}), T-type and L-type ($I_{Ca,L}$). Accordingly, both isradipine and verapamil only blocked the slow $I_{Ca,L}$, which was enhanced by the selective L-type agonist Bay K8644 (Bay). Addition of Elo (10^{-7} M) increased $I_{Ca,L}$ size and specific conductance (G_m/C_m),

by inducing faster activation and inactivation kinetics than control and Bay, and determined a significant negative shift of the activation (V_a) and inactivation curves (V_h), as Bay. These effects resulted potentiated in long-term treated hBCs with Elo (10^{-8} M, 48 h), which also showed increased mRNA and protein expression of pore forming L-Type α_{1C} subunit. In bladder strips from SD rats, Bay induced a dose-dependent increase in tension and its maximal contractile effect was significantly enhanced by Elo-treatment (30 μg/kg per day, 2 weeks). In conclusion, Elo upregulated Ca^{2+} entry through L-type Ca^{2+} channels in human bladder smooth muscle cells, thus balancing its inhibitory effect on RhoA/Rho-kinase signalling, and providing a mechanistic basis for using this drug in the treatment of OAB.

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Vitamin D receptor gene polymorphism influences the amplitude of $1,25(OH)_2D_3$ immune impact

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1,25-Dihydroxyvitamin D_3 ($1,25(OH)_2D_3$) influences the differentiation and cytokine secretion of various immune cell types. These immune modulating effects of $1,25(OH)_2D_3$ are mediated through the nuclear vitamin D receptor (VDR). In the immune system, the $1,25(OH)_2D_3$/VDR complex interacts with promoter vitamin D responsive elements (VDRE), or interferes with the signalling of other transcription factors. A common VDR gene polymorphism is the FokI polymorphism, leading either to a long f-VDR or a shorter F-VDR isoform. In transfection experiments, we investigated whether the long f-VDR and short F-VDR interacted differently with immune transcription factors, such as NFkB, NFAT and AP-1. We also checked whether FokI polymorphism affected the capacity to transactivate a reporter gene driven by a classical VDRE. Finally, the functional impact of the long f-VDR and short F-VDR on immune cell behavior was investigated in monocytes and lymphocytes, which were derived from humans differing in their VDR FokI genotype. The shorter F-VDR resulted in higher NFkB- and NFAT-driven transcription as well as higher IL-12p40 promoter-driven transcription in RAW 264.7 and Jurkat cells ($P<0.05$, ANOVA test and Fisher's LSD multiple comparison). Marginal differences were observed for AP-1-driven transcription and no differential effects were observed for transactivation of a classical vitamin D responsive element for the osteopontin gene. Concordantly, in human monocytes and dendritic cells with a homozygous short FF VDR genotype, expression of IL-12 (mRNA and protein) was higher than in cells with a long ff VDR-genotype. Additionally, lymphocytes with a short FF VDR-genotype proliferated stronger in response to phytohemagglutinin. These data provide evidence that the VDR FokI polymorphism affects immune cell behaviour, with a more active immune system for the short F-VDR, thus possibly playing a role in immune mediated diseases.

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Long-term storage of blood spots: is retesting for newborn 17 OH-progesterone reliable?

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Congenital adrenal hyperplasia (CAH) screening programs are based on 17 OH-progesterone (17OHP) assay in dried blood spots sampled 3d after birth (samples >50 nmol/l are retested as duplicates and the alert threshold is 60 nmol/l). These programs have improved the diagnostic rate of the salt-wasting form. Confronted to delayed hyperandrogenic diagnosis in older children physicians sometimes request retesting years old dried blood spots to rule out erroneous initial true negative screening for CAH. We thus undertook investigation about the reliability of performing 17OHP assays in blood spots up to 10-year-old.

Blotter paper were stored on shelves at room temperature with no autoclaving; samples from odd years from 1997 to 2005 were retested in 2007 (original values distributed from undetectable levels of 17OHP to moderately above recall threshold). No samples from patients with proven CAH were used. Before assays the papers were left 4 days in the laboratory to ascertain uniform inter-sample dryness. 17OHP was assayed using Cisbio assay kit (interassay CV at the alert threshold 7.5%).

We evaluated the variation of 17OHP values between initial and delayed. 17OHP re-assayed in old blood spots was significantly lower than the initial value ($P<0.0001$). A moderate loss of immunoreactivity occured (4 first years) accelerating later on: total decrease in spots aged 2 years −19%, aged 4 years −19%, aged 6 years −24%, aged 8 years −41% Table: median (5%–95%).

	17OHP	Delta (nmol/l)	Delta (nmol/l per year)	Delta (%/year)
1997–2007	68 (37;139)	15.4 (1;33.3)	1.5 (0.1;3.3)	3 (0;5)
1999–2007	54 (27;139)	19.4 (11.6;58.2)	2.4 (1.5;7.3)	5 (3;6)
2001–2007	56 (26;162)	10.8 (−2.5;69.6)	1.8 (−0.4;11.6)	4 (−1;7)
2003–2007	53 (26;121)	9.4 (−0.2;40.2)	2.3 (0.0;10.0)	5 (0;10)
2005–2007	63 (27;140)	9.1 (−3.5;40.5)	4.6 (−1.7;20.3)	9 (−2;19)

The global decrease and the large inter-spot variability of values elicits suspicion about the reliability of re-assaying 17OHP on spots from specific individuals.

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Bioavailable estradiol in men: relationship with age and testosterone

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With age, sex hormones concentrations undergo changes but may continue to have a significant impact: several studies have shown the association of low levels of bioavailable estradiol (BE2) with osteoporosis in man. In postmenopausal women, estradiol levels may influence cognitive function or breast cancer risk. A better approach of sex hormones profiles related to age becomes necessary. We evaluated BE2 concentrations and its variation with age in men, and in postmenopausal women.

Methods

Serum from 183 patients from an endocrinology department: 142 men (15–83) years old, and 41 postmenopausal women, were analysed for measurement of estradiol and BE2. Serum of men were chosen with normal testosterone and bioavailable testosterone (BT) concentrations.

Estradiol concentrations were assayed with a solid-phase RIA (Diasorin) without extraction. Testosterone was measured after extraction using with a solid-phase RIA (IDS). BT and BE2 were obtained after serum equilibration with tritiated hormones, by ammonium sulfate precipitation of SHBG-bound steroid and by the calculation as the product of percentage non-SHBG bound and total estradiol or testosterone concentration.

Results

Total testosterone, BT and BE2 significantly decrease with age (Spearman: $P<0.035$, <0.0001, <0.0001, respectively) as total estradiol does not significantly change (see Table median (5%;95%)).

	TT	T BD	E2T	E2BD
>55 years	18 (8;33)	4.7 (2.6;7.2)	101 (47;183)	47 (21;90)
<55 years	15 (7;27)	3.4 (1.5;5.7)	87 (34;176)	38 (13;86)

BT and BE2 are significantly correlated ($P<0.0001$).

In postmenopausal women median BE2 was16 (8–29) pmol/l.

Conclusion

A BE2 concentration <13 pmol/l in older men (and <8 pmol/l in postmenopausal women) can be considered low. Whether this may constitute a threshold value to be taken into account for assessment of a risk for osteoporosis (or dementia in women) remains to be determined.

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Glucocorticoid receptor gene polymorphisms in patients with Cushing's disease and adrenal Cushing's syndrome

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Introduction
The hypothalamic–pituitary–adrenal axis setpoint and the glucocorticoid sensitivity in various tissues are at least partly genetically determined. The glucocorticoid receptor (GR) gene polymorphisms may have an impact on the development and/or the variability of clinical manifestations of endogenous hypercortisolism, however their role has not been investigated in patients with endogenous hypercortisolism.
Methods
We investigated the potential involvement of the BclI, N363S, ER22/23EK and A3669G polymorphisms of the GR gene in 58 patients with endogenous hypercortisolism (35 patients with Cushing's disease (CD) and 23 with adrenal Cushing's syndrome (ACS). The BclI and the N363S variants were detected by allele-specific polymerase chain reaction, the ER22/23EK by PCR-RFLP method and the A3669G variant by Taqman allelic discrimination assay. Genotype distributions were compared to those measured in 129 healthy control subjects. All patients underwent a detailed clinical and hormonal evaluation, which included measurements of plasma cortisol (at 0800/2400 and after low dose dexamethasone suppression test) and plasma ACTH concentration.
Results
No statistically significant differences were found in the allelic frequencies of the GR gene polymorphisms between patients and controls. The frequency of the BclI polymorphism was underrepresented in patients with ACS compared to healthy subjects but the differences between these groups were not statistically significant ($P=0.066$). None of the studied GR gene polymorphisms were associated with concentrations of plasma cortisol and/or plasma ACTH.
Conclusion
Our findings show that the four investigated genetic variants of the GR gene probably do not involved in the pathophysiology of CD and ACS.

P677

High level of different dietary fat modifies protein androgen receptors level in rat prostate tissues

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The aim of the study was to determine the effect of high-fat diets and dietary fatty acids composition on nuclear androgen receptors protein levels (AR) in rat ventral prostate epithelial and stroma part.
The study was conducted on 30 male Wistar rats with initial body mass 250± 10 g, divided into five groups fed four experimental and one reference diets during three weeks. Experimental semi-synthetic, high-fat (20% w/w) diets were as follows: diet A contained grapes-seed oil reach in PUFA n-6, diet B – lard reach in SFA, diet C – rapeseed oil reach in MUFA n-9 and diet D – fish oil reach in PUFA n-3. Reference rat group (E) was fed low-fat (3% w/w) standard pellets. Animals were kept in individual cages under a 12:12 h light:dark regime, with free access to food and water. After experiment, rats were anesthetized by peritoneal Thiopental injection and sacrificed by cardiac puncture. The ventral prostate was dissected out and immediately fixed in 10% formalin and stored in paraffin blocks. All procedures were approved by the Local Animal Care and Use Committee in Warsaw. AR in rat prostate tissues were determined by immunohistochemisty using polyclonal antibody (PC-167, Calbiochem).
Obtained results showed statistically significant influence ($P\leq0.001$) of dietary fat type on AR in prostate parts. In all experimental groups in comparison to reference group we observed a significantlly lower AR in epithelial part, whereas in stromal part no significant AR changes between all dietary groups were found. Moveover, in group B in comparison to A, C and D groups the highest AR in epithelial parts were stated.
To sumarize, it seems that consumption of high-fat diets can induce protein AR degradation or modify AR gene expression in rat ventral prostate epithelium and that high consumption of SFA increases AR in this prostate tissue.

P678

Association of estrogen receptor alpha and beta gene polymorphisms with sex steroid levels in men

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Estrogens play an important role in male physiology. We investigated the possible association of four single nucleotide polymorphisms in estrogen receptor α (ER1) and Estrogen Receptor β (ER2) genes with circulating levels of sex steroids and Sex Hormone Binding Globulin (SHBG) in men.
SHBG, total and calculated free testosterone (TT and cal FT), estradiol (E2) and free estradiol (FE2) were determined in a population based cohort of 170 apparently healthy Greek men. Body mass index (BMI), waist circumference (WC) and percentage of body fat (%fat) content were measured in all participants. Genotyping for the PvuII and XbaI polymorphisms of the ER1 gene and for the Rsa I and Alu I polymorphisms of the ER2 gene was performed.
PvuII showed an association with E2 levels (median (IQR) pp 58.5 (42.1–73.4) pmol/l versus Pp 48.8 (43–59.6) and PP 57.7 (44.8–67.1), $P=0.032$), and with %fat (median (IQR) pp 24.4 (20.4–27.4) pmol/l versus Pp 22.7 (19.2–25.7) and PP 22.5 (15.3–26.4), $P=0.044$), after adjustment for age and WC. Furthermore, the effect of PvuII on E2 was independent of %fat ($P=0.038$).
A synergistic effect of the 2 ER1 polymorphisms on E2 ($P=0.023$), FE2 ($P=0.03$) and %fat ($P=0.004$) was also present.
A synergistic effect of the ER1 and ER2 genes on TT ($P=0.009$), independent of age, WC and %fat also emerged.
In conclusion, genetic variation in ER1 is associated with estradiol levels and body fat content regulation in men. Furthermore, a synergistic effect of ER1 and ER2 genes is exerted on serum testosterone levels.

Thyroid

P679

Autoimmune thyroiditis (AIT) as a high-risk factor for thyroid cancer (TC)

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We carried out retrospective clinic-statistical analysis of patients' case reports been undergone for surgery.

	1965–74	1975–84	1985–94	1995–2005
Number of operations	1055	2004	3632	3551
TC	8 (0.76%)	41 (2.05%)	140 (3.86%)	266 (7.49%)
AIT	28 (2.65%)	103 (5.18%)	326 (8.89%)	523 (14.73%)

It testifies to increase of TC and AIT within each decade, but pace of TC growth prevails over AIT. Within the last decade 18.42% of TC occurred in combination with AIT. Thus, high percentage of TC on the background of AIT forces to classify patients with AIT as a group of high risk. Such point of view dictates active tactics on a plan of surgery for AIT. Indications for surgery become often difficult and equivocal, because surgery exacerbates autoimmune process, stimulates thyroid degradation, promotes hypothyroidism. Direct correlation between increase of AIT and TC does not enable to reject surgical method of AIT treatment. Basic indications for surgery in AIT: combination of AIT and TC, high risk of pseudo nodes, compression of cervical organs, retrosternal localization, inefficiency of long medication and other non-surgical methods of treatment. Nowadays we prefer radical operations (thyroidectomy/hemithyroidectomy) for AIT. Only in cervical compression syndrome associated with AIT if no discovered nodes, inefficient medication and replacement of thyroid tissue not more for 2/3, we provided isthmus dissection with resection of medial borders of both lobes.
Conclusions
1. Thyroid pathology is changed due to AIT and TC with certain correlation. 2. The main method of AIT management is medication but often it needs surgery. Radical operations are preferable. 3. Pre-operative examination includes all contemporary methods. In doubtful occasions of diseases identification we select surgery.

P680

The influence of cure of subclinical hyperthyreosis on heart rate and autonomous nervous sytem

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Introduction
Subclinical hyperthyreosis (SH) affects about 1.5% population at least. The diagnosis of this disease leans on the laboratory criteria only: decreased of TSH and normal FT3 and FT4 levels. SH increases mortality and there is no unequivocal procedure algorithm to manage patients with this desease.
Aim
To estimate an influence of cure of SH on heart rate and on autonomous nervous sytem and to find the relationships between parameters of Holter electrocardiography (Holter) and of TSH, FT3 and FT4 levels.
Method
About 44 patients (37 women, 7 men) aged 45.9 ± 11, with 12.8 ± 9.8 month history of only autonomous endogenous SH (TSH $= 0.16 \pm 0.1$ IU/l), were examined twice: before and 5.7 ± 4.2 months after TSH normalization (TSH $= 1.32 \pm 0.1$ IU/l) with radioiodine treatment (dose 12.1 ± 5.7 mCi) with the use of Holter and heart rate variability (HRV) evaluation. The average time between examinations was 12.5 ± 6 months. The Local Ethical Committee approval has been obtained.
Results
The cure of SH caused: decrease of mean heart rate ($P = 0.004$), number of ventricular ectopic beats ($P = 0.048$) and dispersion of Q-T interval in ecg (QTd) – $P = 0.02$, and increase of activity of parasympathetic nervous sytem expressed as increase of rMSSD ($P = 0.03$) and LF in horizontal body position ($P = 0.049$). During SH the level of TSH was inversely correlated ($P = 0.048$) but FT3 ($P = 0.017$) and FT4 ($P = 0.004$) were positively correlated with QTd.
Conclusions
1. Cure of autonomous subclinical hypertyhreosis with radioiodine decreases risk of ventricular arrhythmia, and increases activity of parasympathetic nervous system. 2. In autonomous endogenous subclinical hyperthyreosis, the level of TSH inversely and FT3 i FT4 positively correlates with dispersion of Q-T interval in electrocardiogram. 3. Above changes support the decision to treat subclinical hyperthyreosis.

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Radioactive iodide therapy for high-risk papillary thyroid carcinoma

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Radioactive iodide (^{131}I) is an effective treatment modality for papillary and follicular thyroid carcinomas. This retrospective study analyzed the role of ^{131}I therapy in high-risk papillary thyroid carcinoma patients after surgical thyroid removal.
Methods
The study analyzed 1055 consecutive high-risk papillary thyroid cancer patients, including 825 females and 230 males who underwent near-total or total thyroidectomy and follow up at one Medical Center in Taiwan. Patients were categorized into four groups according to treatment outcome. Group A included disease-free patients defined as those with negative ^{131}I whole body scan and lacking serum thyroglobulin (Tg), Tg antibody and recurrence. Group B included non-relapse patients defined as those with no clinical evidence of persistent or recurrent thyroid cancer. Group C included patients with persistent disease defined as those with cancer tissue persisting after surgery. Group D included patients suffering recurrence defined as cancer recurrence after surgery and ^{131}I ablation.
Results
After a mean follow-up period of 10.1 ± 5.4 years (median: 9.5 years), forty-six (4.36%) patients died of thyroid cancer. Nine group A cases with persistent or recurrent cancer were treated until achieving disease-free status. Group C patients received the highest ^{131}I dose but had 25.7% mortality rate. In group D, four of fifty-six (7.1%) patients with recurrent local neck cancer died of thyroid cancer. Conversely, twelve of fifty-six (21.4%) cases died of thyroid cancer with distant metastases.
Conclusions
Radioactive iodide effectively controlled papillary thyroid carcinoma after thyroid ablation in 23.9% of high-risk patients. After surgery and ^{131}I treatments most cases of persistent or recurrent local-regional neck cancer were effectively controlled in a relapse-free status with a cancer mortality rate of 19.0%.

P682

Smoking as a risk factor for thyroid volume change and incident goiter in a region of normalized iodine supply

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Objective
The role of smoking in the pathogenesis of thyroid enlargement is currently under debate. The aim of this paper was to investigate the role of smoking on thyroid volume change and incident goiter for different age-strata in a region with normalized iodine supply.
Methods
The population-based Study of Health in Pomerania (SHIP) compromised 3300 subjects with complete 5-year examination follow-up. Data from 2484 participants without known thyroid disorder and thyroid medication were analyzed. Goiter was assessed by thyroid ultrasound. Determinants of thyroid volume change and goiter were analyzed by linear and logistic regression, respectively.
Results
Participants aged 20–39 years who smoked at baseline and follow-up had a lower risk of incident goiter (odds ratio: 0.39; CI: (0.18; 0.85)). In this subpopulation age was inversely related to thyroid volume change. In the subjects aged 60–79 years smoking at baseline and follow-up was a risk factor for thyroid volume progression (β: 3.19; CI: (0.66; 5.72)). After exclusion of goitrous individuals at baseline this association disappeared.
Conclusion
We conclude that the normalization of iodine supply in this region has led to a decreased impact of smoking on thyroid volume change and incident goiter. Goitrous smokers elder then 60 years did not benefit from the normalized iodine supply.

P683

Hypothyroidism and renal failure

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Renal impairment and electrolyte disorders in hypothyroidism are frequently subtle and rarely observed in clinical practice. Case reports have noted the association but it is rarely found in textbooks. We describe a case of new onset renal dysfunction secondary to hypothyroidism. A 54-year-old male was referred to renal clinic with new renal failure. He described a 3 weeks history of sudden onset peri-orbital, facial and generalised leg swelling and associated muscle ache and pains. He had no significant past medical history and was on no regular medication. On clinical examination he was overweight with marked peri-orbital oedema and facial swelling. Additionally there was bilateral peripheral oedema to the knees. Laboratory results revealed new acute renal failure (ur 10, Creat 200, eGFR 30 ml/min), full blood count and coagulation screen were normal. Bedside urinalysis showed no evidence of blood or protein. Creatine kinase was elevated at 3322 U/l (0–170) with a normal MB fraction and normal electrocardiogram. Cholesterol was elevated at 7, with a normal autoimmune screen and complement level. Renal ultrasonography showed normal kidneys and no abnormalities. Thyroid function tests revealed a TSH of 211.2 and Free T4 of <0.3. TPO antibodies were strongly positive (>600 (0–70)). A diagnosis of autoimmune thyroiditis was made. The patient was commenced on thyroxine replacement, which resulted in resolution of his symptoms and correction of renal function.
The cause of the renal failure in hypothyroidism is due to two mechanisms, decreased renal plasma flow due to a hypodynamic state in hypothyroidism and in severe cases, renal failure can be secondary to rhabdomyolysis. Knowledge of the association between thyroid dysfunction and renal impairment is important for the clinician. We suggest that thyroid function testing should form part of the first line blood investigations for patients with impaired renal function.

P684

Anti-BIP levels on Hashimoto's thyroiditis

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To asses the presence of antibodies to BIP/GRP78, which is a chaperon of endoplasmic reticulum and has immune modulating and antiapoptotic effects in Hashimoto's thyroiditis.
The study included 62 patients with autoimmune thyroiditis. Of these, 20 had euthyroid, 27 had subclinical hypothyroid, and 15 had hypothyroid Hashimato's thyroiditis. A control group including 37 healthy patients also participated in the study.
There was no statistically significant difference between subgroups of Hashimoto's thyroiditis patients and control group, and between subgroups of Hashimoto's thyroiditis patients, individually; when comparison was made in respect of Anti-BIP levels ($P=0.889$).
Although BIP activation has previously been shown, the Anti-BIP level was not different from the control group in subgroups of Hashimoto's thyroiditis patients in our study. This condition suggests that antibodies formed aganist BIP by apoptosis and/or T cell response are not associated with Hashimoto's thyroiditis or that it is at a level in serum that can not be measured.

P685

Neither total thyroidectomy nor radioiodine remnant ablation improved long-term outcome in 900 patients with papillary thyroid microcarcinoma treated during 1945 through 2004

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The study aims were to characterize patients with papillary thyroid microcarcinoma (PTM) and to provide data on long-term outcome. About 900 patients with PTM (tumor size 1 cm or less) had treatment at our centre during 1945–2004. Follow-up extended to 54 years. Mean follow-up for 638 survivors was 13.5 years. Recurrence and mortality details were derived from a computerized database. Median tumour size was 7 mm. About 99% of tumors were grade 1; 98% were not locally invasive. About 30% of patients had nodal metastases at presentation. Three (0.3%) had distant spread at diagnosis. About 85% underwent bilateral lobar resection; regional nodes were removed by either 'node picking' (27%) or an appropriate compartmental dissection (33%). Tumor resection was incomplete in five cases (0.6%). Radioiodine remnant ablation (RRA) was performed in 155 patients (17%). All-causes survival did not differ from expected ($P=0.08$); three patients (0.3%), to date, have died of PTM. None of 892 patients with initial complete tumor resection had distant spread during 20 postoperative years. No localized tumor in a female patient was fatal, and no male patient died of PTM in the first 30 postoperative years. Twenty-year and 40-year tumour recurrence rates were 6% and 9%. About 81% of postoperative recurrences have been in regional neck nodes. Higher recurrence rates were seen with multicentric tumors ($P=0.002$) and node-positive patients ($P<0.001$), but not after unilateral lobectomy ($P=0.49$). Tumor recurrence rates did not appear to be significantly improved by RRA ($P=0.093$). These results reaffirm that papillary microcarcinoma has an excellent prognosis, if primary tumor is completely resected. More than 99% of PTM patients are not threatened by the risks of distant spread or cancer mortality. Neither the performance of a total thyroidectomy, nor the administration of post-operative RRA, improved outcome during 40 years, in terms of either tumor recurrence or cause-specific mortality.

P686

Is response bias in population studies on iodine supply and thyroid disorders neglectable?

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Context
Monitoring of iodine fortification programs is required for early recognition of oversupply or ineffective supply with iodine. Potential effects of non-response studies that are aimed at monitoring such programs have not been analysed.
Objective
With the present analyses we investigated the potential role of selection bias for studies performed to monitor the iodine supply and prevalence of thyroid disorders.
Design
The prospective cohort Study of Health in Pomerania (SHIP).
Subjects
From 4310 baseline respondents, 3949 were eligible for follow-up. Due to nonresponse, 649 subjects were lost. The 3300 follow-up respondents were divided into early and late respondents according to their recruitment level.
Main outcome measure
We used the baseline SHIP population as a hypothesized source population and associated the information on response behaviour from the 5-year follow-up examinations with baseline socio-demographic and thyroid-related characteristics.
Results
We found no significant bias in the prevalence estimates of thyroid-related variables in the comparison between all respondents and the hypothesized source population after response maximization techniques had been applied. Prevalence estimates of several thyroid-related characteristics, however, were biased if we would have only investigated subjects who agreed to participate in our study after the first postal invitation. All differences in thyroid-related characteristics between early respondents and the hypothesized source population were attenuated when analyses were adjusted for socio-demographic variables.
Conclusions
We conclude that the risk of selection bias in prevalence studies in thyroid epidemiology can be diminished by extended efforts put into the invitation procedure. Analyses of risk factors for thyroid disorders should be controlled for determinants of non-response bias that also modulate the risk of thyroid disorders.

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Expression of RNA-binding proteins TIAR and TIA-1 in thyroid tissues from patients with immune and non-immune thyroid diseases

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TIAR and TIA-1 are two closely related RNA-binding proteins, which possess three RNA recognition motifs (RMMs). These proteins are involved in several mechanisms of RNA metabolism, including regulation of mRNA translation. In cellular apoptosis continuously TIAR shuttled between the cytoplasm and nucleus.
The aim of this study was to estimate expression of closely related mRNA binding proteins TIAR and TIA-1 in thyroid tissues from young patients with Graves' disease (GD), toxic (TNG) and non-toxic (NTNG) nodular goiter. Criteria for qualification of Graves' patients: large goiter, ophthalmopathy, TRAb>5, positive titre of anti-TPO and anti-TG antibodies and concentration of TSH <0.3 more the 2–3 months from onset of disease. The analysis of TIAR/TIA-1 expression was performed by western blot and immunohistochemical investigation with DAB-visualization and Mayer's hematoxylin staining.
Identification of mRNA binding protein TIAR in the thyroid follicular cells revealed higher expression of protein in patients with Graves' disease (++) in comparison to patients with NTNG (+/0) and TNG (++/+).The detection of TIAR was presented in thyroid autoimmune disease in bands p44, p39, p36, p31 (kDa), in NTNG in band p44 and TNG in bands p44, p39, p31. TIA-1 molecule identified only in patients with Graves' disease (+) in areas of intrathyroid lymphocytes.

We conclude that alteration in the expression of mRNA binding proteins TIAR and TIA-1 in Graves' tissues may play role in pathogenesis of thyroid autoimmune disease.

P688

Thyroid function and volume changes in patients with end stage renal disease, before and after kidney transplantation

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Background
Disturbance of thyroid hormones metabolism and morphology is common in ESRD (end stage renal disease) but there are discrepancies between data after transplantation. The aim of this study was assessment of the thyroid function and morphology after renal transplantation surgery.
Material and method
All recipient patients, without previous history of thyroid disorders, were enrolled. Serum level of creatinine, total T3 and T4 (TT3, TT4), resin T3uptake (RT3U), TSH and thyroid staging were assessed and also volume and echogenicity were determined by ultrasonography 1 week before and 1, 3 and 6 months after surgery.
Results
Thirty-two patients (22 males, 10 females) with mean $\pm$ S.D. of age 38.2 ± 12.6 years were evaluated. TT3, TT4 and RT3U levels significantly increased by improvement of graft function ($P<0.05$) but values remained in low level in 7 patients with delayed graft function. No case with hyperthyroidism or hypothyroidism was detected. Thyroid volume (mm^3) decreased and echogenicity increased after transplantation ($P<0.05$). Six patients had thyroid nodules and cysts before and 2 new cysts were detected after surgery, that all were benign. There was not any relationship between age, sex, type and duration of dialysis with thyroid function after transplantation.
Conclusion
This survey reveals that there is a clear correlation between thyroid function and morphology (volume and echogenicity) with improvement of kidney function after transplantation but long-term follow-up requires for evaluation of thyroid nodules and malignancies after transplantation.

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Structure-function studies of thyrotropin using site-directed mutagenesis and gene transfer: development of new agonists and antagonists

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Thyrotropin (TSH) and the gonadotropins (FSH, LH, hCG) are a family of heterodimeric glycoprotein hormones composed of two noncovalently linked subunits, α and β. The hTSH heterodimer was converted to a biologically active single-peptide chain (hTSHβCTPα), by fusing the common α subunit to the carboxyl terminal end of hTSHβ subunits in the presence of a $\sim$30 aminoacid peptide from hCGβ (CTP) as a linker. Ligation of the CTP to the carboxyl-end of hFSH resulted in increasing the biological activity and longivity *in vivo*. In addition, the single chain constructs are more active and have longer half-lives compared to dimeric constructs.
The hTSHβCTPα, was used to investigate the role of the *N*-linked oligosaccharides of α and β subunits on secretion and function of hTSH. Two deglycosylated variants were prepared: one lacks both oligosaccharide chains on α subunit (hTSHβCTPα_{1+2}), and the other lacks also the oligosaccharide chain on β subunit of the single chain (hTSHβCTPα(deg)). The single-peptide chain variants were expressed in CHO cells and they are secreted into the medium. Absence of the *N*-linked oligosaccharides on α or β subunits does not affect the secretion of the variants. These results may indicate that the signal for the secretion exists in the single peptide chain and is independent of the oligosaccharides. hTSH variants lack of the oligosaccharide chains is less potent than hTSHβCTPα on cAMP accumulation and T_3 secretion in human cultured thyroid follicles. Moreover, both deglycosylated variants compete with normal hTSH and human thyroid stimulating immunoglobulin (hTSI) in a dose dependent manner and decreased significantly the hTSH and hTSI-stimulated levels of cAMP and T_3 secretion. Thus, this variant, behaves as potential antagonist, who may offer a novel therapeutic strategy in the treatment of Grave's disease, the most common form of hyperthyroidism.

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Clinicopathological characteristics of thyroid cancer in patients on dialysis for end-stage renal disease

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Objective
Our aim was to determine the prevalence and clinicopathological characteristics of thyroid cancers in the dialysis population and to evaluate potential risk factors.
Design
We performed a retrospective analysis of our end-stage renal disease (ESRD) patients on dialysis and thyroidectomized patients without ESRD (2000–2006). Then we compared the data of thyroid cancer patients on dialysis ($n=9$) with the data of patients who had histopathologically verified benign thyroid disease on dialysis ($n=23$) and with the histopathological data of thyroid cancer patients without ESRD.
Main outcome
Papillary thyroid cancer (PTC) was the only histotype which was found in 9 of 420 (2.1%) ESRD patients on dialysis. Multifocal PTC was found in 8 of 9 patients, of them 4 were follicular variant of PTC (FVPTC). Two patients had lymphatic metastasis at diagnosis. Eight PTCs were classified as TNM stage I and one as stage II. Among the analyzed factors, age ($r=0.374$, $P=0.01$) and duration of dialysis ($r=0.436$, $P=0.007$) showed a significant positive correlation with the occurrence of thyroid cancer.
Conclusions
We conclude that the prevalence of thyroid cancer in patients undergoing dialysis was not higher than background population. Age and duration of dialysis showed a significant positive correlation with the occurrence of thyroid cancer in patients on dialysis. Among the histotypes, there may be higher percentage of PTC, FVPTC and multifocality in dialysis patients. The effect of these characteristics on prognosis of thyroid cancer in dialysis patients is needed to be further evaluated.

P691

The TRβ1 is essential in mediating T3 action on Akt pathway in human pancreatic insulinoma cells

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Thyroid hormone action, widely recognized on cell proliferation and metabolism, has recently been related to the phosphoinositide 3 kinase (PI3K), an upstream regulator of the Akt kinase and the involvement of the thyroid hormone receptor β1 has been hypothesized. The serine-threonine kinase Akt can regulate various substrates that drive cell mass, proliferation and survival. Its action has also been characterized in pancreatic β-cells. We previously demonstrated that Akt activity and its activation in the insulinoma cell line hCM can be considered a specific target of the non genomic action of T3. In this study, we analyzed the molecular pathways involved in the regulation of cell proliferation, survival, size and protein synthesis by T3 in a stable TRβ1 interfered insulinoma cell line, derived from the hCM, and evidenced a strong regulation of both physiological and molecular events by T3 mediated by the thyroid hormone receptor β1. We showed that the thyroid receptor β1 mediates the T3 regulation of the cdk4·cyc D1·$p21^{CIP1}$·$p27^{KIP1}$ complex formation and activity. In addition TRβ1 is essential for the T3 upregulation of the Akt targets β-catenin, p70S6K and for the phosphorylation of Bad and mTOR. We demonstrated that the β1 receptor mediates the T3 upregulation of protein synthesis and cell size, together with the cell proliferation and survival, playing a crucial role in the T3 regulation of the PI3K/Akt pathway.

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Rosiglitazone in two patients with thyroglobulin-positive and radioiodine-negative differentiated thyroid cancer

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Thiazolidinediones have demonstrated some efficacy in promoting radioiodine uptake of previously poorly radioiodine-responsive thyroid cancers. We describe the effect of rosiglitazone, in promoting uptake in thyroid metastatic tissue with weak uptake in order to treatment with radioiodine in two patients.
Case 1
A 62-year-old woman with thyroid cancer and negative I-131 body scan post therapy (150 mCi) with presence of recurrent disease (PET positive in neck, pulmonary and mediastinum and persistently elevated serum thyroglobulin concentrations). The patient received 8 mg rosiglitazone daily for 3 months. Serum thyroglobulin stimulated before initiation of therapy with rosiglitazone was 37.5 ng/ml. After differentiation treatment, she received 150 mCi I-131 and the posttherapy scan showed marked uptake in the neck and Tg elevation after rTSH to 172.4 ng/ml in contrast to the predifferentiation treatment scan.
Case 2
A 63-year-old woman with thyroid cancer and negative I-131 body scan post therapy (150 mCi) with presence of recurrent disease (PET positive in neck and mediastinum and serum antibody antithyroglobulin elvated 395.8 UI/ml). The patient received 8 mg rosiglitazone daily for 3 months. After differentiation treatment, she received 150 mCi I-131 and the posttherapy scan showed marked uptake in the neck and Ac-antTg elevation to 436.1 UI/ml.
Both patients tolerated treatment well without symptoms of hypoglycemia or change in liver function tests.
Conclusion
Our two patients showed uptake augmentation of I-131 after pretreatment differentiation with rosiglitazone. It might encourage the use of TZDs in patients with radiotherapy resistant differentiated thyroid cancers.

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Effects of 8-Cl-cAMP on growth and apoptotic process in poorly differentiated thyroid cancer cell lines

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Tools that are highly effective in the treatment of differentiated thyroid cancer (DTC) loose their therapeutic potentials in poorly differentiated tumors. The synthetic analog 8-Cl-cAMP has been known to have an antiproliferative effect in a variety of cancer cells and is tested as antineoplastic agent in clinical trials. The signaling mechanisms that govern the 8-Cl-cAMP-induced growth inhibition are still uncertain and data in thyroid neoplasia are lacking. Therefore, we tested the effects of 8-Cl-cAMP on the growth and apoptotic process in anaplastic (ARO), papillary (NPA) and follicular (WRO) thyroid carcinoma cell lines. Our proliferation data show that growth of ARO, NPA and WRO was inhibited by more than 50% after the treatment with 8-Cl-cAMP in a time- and dose-dependent manners. To test whether apoptosis occurs in 8-Cl-cAMP treated cells, we analyzed cell cycle, DNA fragmentation and caspase activity. We found induction of apoptosis in all the cell lines with different sensitivities. Since MAPKs are involved in the regulation of proliferation and apoptosis, we investigated modification of ERKs, that are preferentially activated in response to mitogens, and p-38 MAPKs, that are activated in response to cell stresses. Following the treatment with 8-Cl-cAMP, no modification of ERK phosphorylation was found while a marked and progressive induction of p38-MAPK phosporylation was seen in all the cell lines. We also evaluated the Akt phosphorylation, as a marker of the PI3K proliferative pathway that as been implicated in thyroid cell proliferation, and we found poor modifications of Akt phosphorylation state after 8-Cl-cAMP treatment.
In conclusion, 8-Cl-cAMP has a potent inhibitory effect on WRO, NPA and ARO cell growth which is accompanied by a pro-apoptotic effect via p38-MAPK. Therefore, 8-Cl-cAMP has a potential to be tested *in vivo* as a therapeutic agent for poorly DTC.

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Cyclin A and B1 are overexpressed in thyroid cancers which had been operated for indeterminate, nondiagnostic, or suspicious fine-needle aspirate results

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Objective
Approximately 30% of patients with thyroid nodules have indeterminate, nondiagnostic, or suspicious fine-needle aspiration (FNA) biopsy results. These patients usually undergo thyroidectomy because of cancer risk. Our aim was to determine diagnostic markers to distinguish benign from malignant thyroid neoplasms, so we focused on G2-M boundary regulators of the cell cycle and investigated the expression of two proteins, cyclin A and cyclin B1.
Methods
We studied the expression of cyclin A and B1 in resection specimens of 168 indeterminate, nondiagnostic, or suspicious FNA biopsy results retrospectively using immunohistochemistry.
Results
Sixty-four of resection specimens were consisted of malignant histopathology. Of 64 cases of thyroid cancer (58 papillary, 4 follicular, 1 medullary, and 1 hürthle cell carcinoma), cyclin A was overexpressed in 33 cases (51.5%) in contrast to 33 cases (31.7%) of 104 benign pathology specimens ($P=0.025$). Tweny-five (39.1%) of thyroid cancer cases were positive for cyclin B1 in contrast to 16 (15.4%) of 104 benign cases ($P=0.001$). Cyclin A overexpression was not linked to cyclin B1 overexpression. No association was found between overexpressions of cyclin A, cyclin B1 and TNM stage in malignant cases.
Conclusions
In this study, we have demonstrated that cyclin A and B1 may be important biomarkers in predicting malignancy in indeterminate, nondiagnostic, or suspicious FNA biopsies. The use of these biomarkers may allow an accurate preoperative diagnosis of thyroid cancer. The value of these biomarkers warrants further evaluation in a prospective study on fresh cytological samples of indeterminate, nondiagnostic, or suspicious FNA biopsies.

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Semiquantitative evaluation of E-cadherin immunoexpression in the thyroid neoplasms

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Cadherins are proteins important for regulation of cell to cell adhesion. It has been postulated that the loss of cadherins may be an essential step in progression of cancer cell dedifferation, leading to increased metastatic potential. In the present study, the immunoexpression of E-cadherin was examined in various types of thyroid neoplasms and compared with that expression in normal thyroid tissue. The histopathological slides from 138 thyroid carcinomas (90 papillary, 8 follicular, 15 oxyphilic cell type, 14 poorly differentiated, and 11 undifferentiated), 16 follicular adenomas and 26 normal thyroids were immunostained with the use of anti E-cadherin antibody. The intensity of staining was assessed semiquantitatively by evaluation of 1000 cells in each lesion. The results were expressed in an ordinal scale from 0 to 4 (0 – staining present in $<5\%$ of cell, 1 – 6–30% of cells, 2 – 31–60% of cells, 3 – 61–90% of cells, and 4 – more than 90% of cells). Statistical significance was tested with Mann-Whitney *U* test. The lowest mean expression of E-cadherin was found in undifferentiated carcinomas (1.63) and the second lowest (2.07) – in poorly differentiated carcinoma. All other examined lesions showed significantly higher expression of E-cadherin (the highest expression was observed in follicular adenomas – 3.25 – $P<0.005$). There was no significant difference between undifferentiated and poorly differentiated carcinomas. Interestingly, there was a significant difference in immunoexpression of E-cadherin between follicular adenoma and papillary carcinoma (2.47, $P<0.05$) as well as normal thyroid tissue (2.69, $P<0.05$). The correlation between TNM classification and the level of E-cadherin immunoexpression was also examined. In the group of poorly or undifferentiated carcinomas there was a very weak negative correlation between the lymph nodes involvement and the level of E-cadherin immunostaining (Spearman coefficient $=-0.25$). No such correlation was observed in the groups of differentiated carcinomas. There was no correlation between the presence of metastases and the E-cadherin expression in none of the examined groups of cancers.

Conclusions
The loss of E-cadherin expression can be observed in poorly differentiated and undifferentiated carcinomas. However, it seems that prognostic value of E-cadherin immunoexpression is rather weak.

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Pronostic value of serum thyroglobulin measured before thyroid ablation with I^{131} in patients with papillary cancer meta-analysis

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Objective
To know the pronostic value of serum thyroglobuline (Tg), obtained before thyroid ablation with I^{131}, in order to predict cancer recurrence risk in patients treated with total thyroidectomy due to differentiated thyroid carcinoma (DTC).
Methods
We conducted a computed search of the published literature in Medline. We included diagnostic test studies that evaluated the relationship between Tg before I^{131} ablation, with local or distance tumoral recurrence. Selection criteria were Tg measurement method description and the explicit exclusion of patients with positive thyroglobuline antibodies. To express the accumulate diagnostic test pronostic value, we applied likelihood ratio (LR). To obtain accumulate likelihood ratio we introduced our data in the Revman 4.2 program (Cochrane Initiative) and selected the accumulated OR function. Statistical heterogeneity across the studies was tested with the use of Cochrane Q statistic and Higgins's I^2 coefficient in a randon effect model.
Results
The mean recurrence rate was 20.39%. Next table shows accumulate LR by Tg value ranges:

Tg (ng/ml)	LR+	CI 95%	LR−	CI 95%
2.0−2.25	6.8	3.9−11.8	0.2	0.1−0.3
5.0−5.05	9.7	5.9−15.9	0.1	0.06−0.17
10.0−11.05	16.2	9.9−26.1	0.06	0.04−0.10
27.5−30.25	38.8	20.2−74.3	0.03	0.01−0.05
37.5−38.10	39.3	22.3−69.3	0.03	0.01−0.04

Conclusions
The best range of values of Tg before I131 ablation in order to predict recurrence in DTC's thyroidectomized patients is 27.5–30.25 ng/ml.
For a recurrence rate of 20.39% the positive predictive value of Tg before ablation is 91% and the negative predictive value 0.76%.

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Clinical features of TRAB negative Graves disease: a prospective study

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Objective
It is well-established that hyperthyroidism of Graves disease is caused by generation of TSH-receptor stimulating autoantibodies. However, in some patients TRAB titers may be in normal levels. The rate and the burden of TRAB negative disease vary between studies. In this study, we compared the clinical and laboratory parameters of 170 patients (85 TRAB positive and 85 TRAB negative) that were diagnosed to have Graves disease in our department.
Methods
Diagnostic criteria for Graves disease; i) Elevated free T3 or free T4 ii) Typical thyroid inferno pattern in ultra-sonography iii) Elevated radioiodine uptake (I-131). TRAB was measured with radioreceptor assay (RRA)-(RIAZEN-Belgium).
Results
Mean age of study group was 46.7 and female dominance was present (72%). FT4, FT3, ATPO, thyroid volume and radioiodine uptake was significantly elevated in TRAB positive disease. Mean age or the presence of thyroid nodules did not differ between groups. TRAB negative disease was more common in females ($P=0.06$). Thyroid ophtalmopathy was diagnosed dominantly in TRAB positive disease (41% vs %24 $P=0.05$). The recurrence of disease in radioactive-iodine or anti-thyroid drug treated subjects was more common in TRAB positive subjects (%25 vs %7). TRAB titers were found to be correlated with FT4, FT3, ATPO, thyroid volume and radioiodine uptake.
Conclusion
TRAB negative Graves disease is not a clinical entity on which the endocrinologists have fully agreed. It has been suggested that technical difficulties in TRAB assays might contribute to lower levels. Furthermore, some authors suggest that Hashitoxicosis should be the proper diagnosis in patients with TRAB negative thyrotoxicosis.
Our results demonstrate that TRAB negative Graves disease is associated with a milder form of thyrotoxicosis. However, either the clinical presentation of our patients or their laboratory data are distinguishable from Hashimato Thyroiditis. We suggest that TRAB negative Graves disease is a real clinical subtype of Graves disease. It must be kept in mind that, despite low TRAB levels, ophtalmopaty or recurrence of disease may occur.

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Absence of sonic hedgehog mutations in a large cohort of children with thyroid dysgenesis

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Thyroid dysgenesis accounts for 75% of all cases of congenital hypothyroidism (CH), and includes thyroid agenesis or hemiagenesis, thyroid hypoplasia, and thyroid ectopy. Thyroid transcription factors TTF-1, TTF-2 and Pax-8, which are involved in the development of the thyroid gland and its normal migration, have been indicated as the best candidate genes but have been found to be mutated in a minority of cases. Sonic Hedgehog (Shh) protein is involved in several key events during vertebrate embryogenesis, as well as left-right axis determination and organ development. In mice, Shh is expressed in cardiac bud and indirectly governs the symmetric bilobation of the thyroid. Shh knockout mice develop a single unilateral thyroid mass and ectopic thyroid tissue remnants from the presumptive trachea. The aim of this study was to investigate if mutations of *SHH* gene could be implicated in human thyroid dysgenesis.
The *SHH* gene has been analyzed in 23 cases of CH associated with thyroid ectopy. Three cases were of particular interest due to a particular dysgenetic phenotype: sublingual ectopy was associated with a thyroglossal duct cyst in one and with hemiagenesis in the second, the third patient was euthyroid with an *in situ* thyroid gland and an incidentally discovered intracardiac thyroid ectopy. The three coding exons of *SHH* have been amplified and directly sequenced, but no mutations have been detected. A novel silent polymorphism at codon 299 (CTG→CTC) has been found in one patient with isolated thyroid ectopy.
In conclusion, no germline mutations of *SHH* gene have been found in a large group of children with CH and thyroid ectopy. Though a key role in mouse thyroid development has been proposed, SHH defects may be rarely involved in the pathogenesis of isolated thyroid dysgenesis in humans.

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Thymic hyperplasia presenting as a neck mass in Graves' disease

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Introduction
Thymic hyperplasia is a rare manifestation of Graves disease. In this report, we describe a female with Graves' disease and a neck mass that was associated with thymic hyperplasia.

Case report
A 28-year-old woman was referred to our division for the evaluation of palpitations. Thyroid function tests were associated with thyrotoxicosis. Thyroid receptor antibody (TRAb) was found to be 22.3 U/l (positive: >14 U/l). Sonography showed bilateral enlargement of thyroid lobes, typical 'thyroid inferno' pattern and also a homogenous mass of 10×15×25 mm in dimensions, at the inferior of the thyroid. Magnetic resonance imaging demonstrated that the mass was a homogenous anterior mediastial lesion plunging to the neck, compatible with hyperplasia of the thymus. Thymic hyperplasia related to GD was suggested and follow up was recommended. She was treated with metimazole. At the 3rd month of therapy euthyroidism was obtained but there was no significant change in either the size or the sonographic characteristics of the mass. At the 6th month of therapy neck ultrasonography did not reveal any regression of the mass. Total thyroidectomy and mass extirpation were performed and pathological examination was consistent with chronic lymphocytic thyroiditis and thymic hyperplasia.
Conclusion
Elevations in circulating thyroid hormones are suggested to cause thymus hyperplasia in Graves disease. It must be noted that microscopic changes in the thymus can be detected in one third of patients but massive enlargement is rare. This presented patient has two different features when compared with the previously reported cases. First, she presented with a neck mass instead of a mediastinal mass. This is probably due to a different growth pattern of thymus from mediastinum towards the cervical area Second, the hyperplastic thymus did not regress after anti-thyroid treatment. We suggest that additional factors rather than the level of circulating thyroid hormones may be involved in the pathogenesis of thymic hyperplasia in Graves' disease.

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Cigarette smoking but not the TSHR germline polymorphism D727E is associated with toxic multinodular goitre (TMNG) and the thyroid volume

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Objective
There are contradictory results regarding the possible association of the TSHR polymorphism D727E with TMNG. Furthermore the influence of smoking on the thyroid volume has been reported by several authors. A possible association of smoking with thyroid nodules and particularly TMNG has not been investigated up to date.
Methods
In this study, 88 patients with TMNG were included. Diagnosis was verified by ultrasonography, scinthiscan, and measurement of TSH, fT4 and fT3. Patients were age- and sex-matched to 88 controls without any thyroid disorders. The polymorphism was detected by denaturating gel electrophoresis (DGGE).
Results
The prevalence of polymorphism D727E in the TMNG population (23.9%) did not deviate significantly ($P=0.355$) from the control group (18.2%). There was no significant ($P=0.601$) difference for thyroid volumes between polymorphism carriers (PC) (16.5 ml) and wildtype probands (WP) (18.5 ml). Moreover, also within the TMNG group ($n=88$) and the control group ($n=88$) there was no significant (TMNG: $P=0.603$; controls: $P=0.332$) difference for thyroid volumes between PC and WP (TMNG: PC 36 ml, WP 36.5 ml; controls: PC 13.9 ml, WP 11.6 ml). Compared to nonsmokers ($n=132$, 44.7%) the prevalence of TMNG was significantly higher ($P<0.05$) for smokers ($n=44$, 65.9%) in the entire population (TMNG and controls). Smokers also had a significantly higher thyroid volume than nonsmokers in the entire population (TMNG and controls, $n=176$) (smokers: 34 ml, nonsmokers: 15.7 ml, $P<0.05$). Moreover, also within the TMNG group thyroid volumes of smokers (43 ml) differed significantly from nonsmokers (31 ml, $P<0.05$) and in the control group smokers tended to have a higher thyroid volume (smokers: 15.8 ml, nonsmokers: 11.6 ml, $P=0.119$).
Conclusion
Neither an association of the polymorphism with TMNG nor its association with the thyroid volume was evident in this study. However, smoking clearly showed a positive association with the thyroid volume. Furthermore, our results suggest an association of smoking with TMNG.

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Thyronamines are isozyme specific substrates of deiodinases

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3-iodothyronamine (3-T_1AM) and thyronamine (T_0AM) are novel endogenous signaling molecules that exhibit great structural similarity to thyroid hormones but apparently antagonize classical thyroid hormone (T_3) actions. Their proposed biosynthesis from thyroid hormones would require decarboxylation and more or less extensive deiodination. Deiodinases (Dio1, Dio2 and Dio3) catalyze the removal of iodine from their substrates. Since a role of deiodinases in thyronamine biosynthesis requires their ability to accept thyronamines as substrates, we investigated whether thyronamines are converted by deiodinases.
Thyronamines were incubated with isozyme specific deiodinase preparations. Deiodination products were analyzed using a newly established method applying liquid chromatography and tandem mass spectrometry (LC-MS/MS). Phenolic ring deiodinations of 3,3′,5′-triiodothyronamine (rT_3AM), 3′,5′-diiodothyron-amine (3′,5′-T_2AM), 3,3′-diiodothyronamine (3,3′-T_2AM) as well as tyrosyl ring deiodinations of 3,5,3′-triiodothyronamine (T_3AM) and 3,5-diiodothyronamine (3,5-T_2AM) were observed with Dio1. These reactions were completely inhibited by the Dio1 specific inhibitor 6n-propyl-2-thiouracil (PTU). Dio2 containing preparations also deiodinated rT_3AM and 3′,5′-T_2AM at the phenolic rings but in a PTU-insensitive fashion. All thyronamines with tyrosyl ring iodine atoms were 5(3)-deiodinated by Dio3 containing preparations. In functional competition assays, the newly identified thyronamine substrates inhibited an established iodothyronine deiodination reaction. By contrast, thyronamines which had been excluded as deiodinase substrates in LC-MS/MS experiments, failed to show any effect in the competition assays, thus verifying the former results.
These data support a role for deiodinases in thyronamine biosynthesis and contribute to confining the biosynthetic pathways for 3-T_1AM and T_0AM.

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The outcome of radioiodine therapy of hyperthyroidism: comparison of patients with a toxic nodular goiter and with Graves' disease

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Introduction
Hyperthyroidism is one of frequently encountered clinical syndromes appearing in about 2% of adult population. The aim of this study was to evaluate the efficacy of radioiodine treatment in relation to the form of hyperthyroidism.
Material and methods
The study investigated 300 patients: 150 with Graves' disease and 150 with a toxic adenoma goiter (109 with a solitary nodule and 41 with a multinodular goiter). In all the cases, the estimation of FT3, FT4, TSH, TSI concentrations, radioiodine uptake and technetium-99m pertechnetate scans were carried out. The radioiodine dose was calculated on the basis of Marinelli's formula.
Results
After a year long observation period, 31.35% of the patients with Graves' disease were found to be euthyroid, 31.35% hypothyroid and 37.3% hyperthyroid, whereas among the patients with a toxic nodular goiter 84% were euthyroid, 2% hypothyroid, and 14% were hyperthyroid. The results of radioiodine therapy for a solitary nodule and a multinodular goiter were similar.
Conclusion
The radioiodine therapy revealed a significantly higher efficacy with a lower rate of hypothyroidism in the therapy of a toxic nodular goiter than in Graves' disease, but its efficacy in the patients with a solitary nodule and a multinodular goiter was comparable.

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Results of thyroxine therapy on thyroid nodules size in children with Hashimoto's thyroiditis

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Aim
To study the effect of thyroxine treatment on the size of thyroid nodules in children with Hashimoto's thyroiditis
Patients and methods
Twenty-two girls and 6 boys (78.6/21.4%) were followed for 2 years under thyroxine therapy, at mean dose 1.6 ± 0.4 μg/kg per day. Diagnosis of Hashimoto's thyroiditis was based on the high value of either antithyroid antibodies, ATPO or ATG. Age at presentation was 10.6 ± 2.0 year (6.2–13.6). Nineteen patients were pubertal and 9 prepubertal (67.9/32.1%). Thirteen (46.4%) were euthyroid, 12 (42.9%) with subclinical hypothyroidism and 3 (10.7%) hypothyroid. The size of thyroid nodules was 0.97 ± 0.38 cm (0.6–2.2). All nodules ≥ 1 cm were examined by FNA that confirmed Hashimoto's thyroiditis. For the statistical analysis, *t*-test and Mann–Whitney test were used.
Results
At presentation, there was not any significant difference in nodule size between euthyroid children and children with hypothyroidism or subclinical hypothyroidism, 0.9 ± 0.2 vs 1.0 ± 0.4 cm, respectivelly, $P > 0.05$. Nodule's size ≥ 1 cm had 42.9% of children ($N = 12$). Decrease in nodule size ≥ 0.3 cm after 2 years with thyroxine was observed in 75.0% ($N = 21$). Overall, nodule size showed significant decrease, from 0.97 ± 0.38 cm at start of thyroxine to 0.64 ± 0.48 cm, $P < 0.001$, after 1 year and to 0.65 ± 0.47 cm, $P < 0.001$, after 2 years of treatment. Separately, in euthyroid children nodule size decreased from 0.96 ± 0.30 cm at start of thyroxine, to 0.58 ± 0.48 cm, $P = 0.027$, after 1 year and to 0.63 ± 0.46 cm, $P = 0.045$, after 2 years under treatment. In children with hypothyroidism or subclinical hypothyroidism, nodule size decreased from 1.00 ± 0.43 to 0.69 ± 0.49 cm, $P = 0.024$, after 1 year and to 0.68 ± 0.49 cm, $P = 0.007$, after 2 years under treatment.
Conclusions
Treatment with thyroxine in children with thyroid nodules due to Hashimoto's thyroiditis can significantly decrease the size of the nodules, in children with euthyroidism and in those with hypothyroidism or subclinical hypothyroidism, as well.

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Refetoff syndrome associated with other congenital diseases (case report)

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We report the case of a 25-year-old woman (K.Cs.) hospitalized in our clinic for endocrinological investigation, presenting a spontaneous abortion 3 weeks before hospitalization. From medical history, we mention a cerebral thrombophlebitis with intracranian hypertension syndrome, appeared after oral contraceptive drug (Diane 35^{R}) used for 5 months. The clinically asymptomatic patient presented diffuse goiter (I. gr.), overweight and virilism, without other clinical signs (pulse 68/min). The hormone determinations showed an elevated TSH-level (6.43 mIU/l, normal range: 0.44 – 3.45), free T_4 at the upper normal limit (1.94 ng/dl, normal range: 0.8–2.0), and T_3 close to the upper normal value (3.4 ng/ml, normal range: 2.02 – 4.43). The repeated investigations gave similar results. Based on these data and on the clinical euthyroidism, we suspected a thyroid hormone resistance syndrome. The TRH-stimulation test (200 μg i.v.) showed an increased response to thyrotropin: the basal TSH (4.63 mIU/l, normal range: 0.44 – 3.45) increased after 20 min to 25.69, being high also after 40 and at 60 min (24.1 and 25.01 mIU/l, respectively). This result and the negative cranial MRI excluded the possibility of a hypophyseal TSH-secreting adenoma. Similarly, the lack of clinical signs of hypothyroidism and FT_4 at the upper normal limit excluded the possibility of a deficient transmembrane transport of thyroid hormones, due to a mutation of the MCT8-gene (mono-carboxylate transporter-8). At the same time, this syndrome has been described only in the men, women being just carriers of the mutation. The T_3 suppression test and other laboratory examinations are in course. Besides the Refetoff syndrome the patient was diagnosed with catastrophic antiphospholipidic syndrome (incomplete form, having only 2 criteria present), although the levels of antibodies weren't high (IgG anti-cardiolipin antibody, anti-DNAds, antiSm/RNP antibody, cryoglobulines absent). Further investigations detected a congenital hypercoagulability syndrome, too.

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Thyroid function of pregnant women hospitalized in a section of gestational pathology

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In our previous studies, we determined the incidence of thyroid dysfunctions in pregnant women in endocrinological out-patient departments, comparing the frequency and nature of gestational complications in hypothyroid pregnant women versus pregnants without thyroid disturbances. In the present study, we proposed to appreciate thyroid dysfunctions in a clinical section of pregnant women with gestational pathology. We investigated 75 pregnant women hospitalized in the Clinic of Obstetrics and Gynecology Nr. I. Tg.Mureş, gestational pathology section, during September–November 2006. We determined the TSH in all women, and in addition FT_4, T_3 and anti-TPO antibodies in those patients whose gestational complications couldn't be explained by obstetrical–gynecological (placental or organic genital pathology), general or acute infectious diseases. In 11 cases (14.7%), we obtained pathological hormone values, detecting in 6 women (8%) hypothyroidism and in 5 (6.7%) hyperthyroidism. Among these patients 10 were hospitalized for serious gestational complications, without any other trigger factors. In half of the women with hypothyroidism (3), we observed a normal TSH-level with a decreased FT_4-value, situation described by other authors in 2/3 of pregnant women. From the 6 hypothyroid women 5 (83.3%) presented considerable gestational complications: those 3 with normal TSH had tardive dysgravidia, imminent abortion or history of precocious neonatal mortality, while the other 2 were hospitalized for imminent abortion and pregnancy induced arterial hypertension, respectively. All the 5 hyperthyroid women presented serious gestational complications: 2 imminent abortion, one imminent premature delivery, one gestational hyperemesis and a woman had 3 spontaneous abortion in anamnesis. In conclusion, among the 44 pregnant women having gestational complications with unknown etiology, in 11 (25%) was detected a thyroid dysfunction.

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Assessment of cobalt status: a comparison between goiterous children and healthy control subjects

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Introduction
Cobalt is a relatively rare magnetic element with properties similar to iron and nickel. Cobalt is an essential element necessary for the formation of vitamin B12; however, excessive administration of this trace element produces goiter and reduced thyroid activity. This study was done to compare the prevalence of cobalt excess (as a cause of goiter) between goiterous and non goiterous children and to assay the relationship between serum cobalt and thyroid hormones too.
Methods
This cross sectional study was carried out on 5380 randomly selected children aged 8–11 years. Anthropometric measurements and thyroid exam were done on all of them. Serum concentration of T_4, T_3, thyroid stimulating hormone (TSH), serum cobalt and urine iodine were analysed from a subsample of 169 goiterous children who were selected randomly. All above data were collected in an age and sex matched control group too.
Results
The serum cobalt level was significantly lower in case group (4.3 ± 2.9 μg/l in case versus 6.3 ± 2.7 μg/l in control group ($P < 0.0001$)).The urine iodine level was significantly lower in case group. (19.8 ± 10.9 μg/l in case versus 25.8 ± 10.9 μg/l in control group ($P < 0.0001$)).There was 12(7.1%) cases of cobalt deficiency and no one has cobalt excess. There was not a significant difference between Cobalt deficiency and goiter ($P = 0.07$), but there was a significant difference between iodine deficiency and goiter ($P = 0.01$). The total goiter rate was 34.8%(G_{II}) and 100% of cobalt deficient children were goiterous. There was not a significant correlation between cobalt concentration and height, weight, BMI, T_4, TSH and urine iodine, but there was a weak correlation with T_3 ($P = 0.04$, $r = 0.1$).
Discussion
This study shows that goiterous children had lower serum cobalt concentration. In most of previous studies, cobalt excess was known as a cause of goiter. Further studies for detecting the effect of cobalt replacement on goiter size may be needed to establish the cause and relationship between cobalt deficiency and goiter.

Conclusion
Serum concentration of cobalt and urine iodine was lower in goiterous children and it may be a cause of goiter in our region (Kerman–Iran).

P707

Lack of apparent association of TSH receptor mutations *in vitro* activity with the clinical course of patients with sporadic non-autoimmune hyperthyroidism

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Up to date, 12 patients with sporadic non-autoimmune hyperthyroidism (SNAH) caused by sporadic germline mutations in the TSHR gene have been reported. Nearly, all case reports discussed possible associations of the TSHR mutations *in vitro* activity (IVA) with the clinical course (CC). Therefore, we analyzed this question in a systematic review of the case reports and investigated the TSHR mutation's IVA in selected cases.
Recently, linear regression analysis (LRA) of constitutive activity as a function of TSHR expression determined by 125I-bTSH binding or FACS analysis compared to the wt TSHR was described as a more reliable way of characterizing the IVA of a constitutively activating TSHR mutation. Therefore, we determined the LRAs for all sporadic germline mutations which had not previously been reported. Moreover, we systematically evaluated all case reports of SNAH for evidence of an association of the CC with the IVA of the mutated TSHR. The LRAs determined were: M453T (5.2 ± 0.8), L512Q (4.5 ± 0.7), I568T (25.6 ± 6.3), F631L (45.9 ± 9.4), T632I (14.5 ± 2.7), D633Y (16.4 ± 6.4). Only 4 of 10 investigated clinical signs namely prematurity, early onset of goiter (<15 months), eye signs (proptosis and eyelid retraction) and craniosynostosis are associated with a high LRA (>10). Furthermore, mental retardation, craniosynostosis and eye signs are associated with an early onset (<1.5 months) and a long duration (>3 years) of insufficiently treated hyperthyroidism. The comparison of the CCs of patients harboring the same mutation (M453T, S505N) showed no relation of the clinical activity with a high LRA.
Considering the different diagnostic circumstances, therapeutic strategies and the limitations of a systematic analysis of case reports due to the restricted number of case reports and limited follow-up we found no consistent relation of the TSHR mutation's IVA determined by LRA with the CC of patients with SNAH.

P708

Phenotypic profiling of MCT8 deficient mice

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Thyroid hormones are essential for the proper development of a variety of tissues, especially the nervous system. Their transport into target cells is mediated by specific thyroid hormone transporters like the monocarboxylate transporter 8 (MCT8). Mutations in this X-chromosomal gene in humans lead to a severe phenotype characterized by psychomotor retardation, hypotonia, and a striking derangement of serum thyroid hormone levels: high T3 in the presence of low T4, and no significant changes in TSH. Interestingly, despite high serum T3 the patients do not show tachycardia. Here, we present data on the expression of MCT8 in various human and mouse tissues, as well as on clinical phenotypes of MCT8 knock-out mice. To this end, we submitted MCT8-deficient mice to a comprehensive phenotypical screen at the German Mouse Clinic, located at the GSF in Munich. MCT8-deficient mice replicated the hormonal phenotype of the patients, but did not exhibit neurological deficits and hypotonia. No obvious neuroanatomical changes were observed in MCT8 knock-out mice as analyzed by immunohistochemistry. Nevertheless, the loss of the transporter causes behavioral changes that need further investigation. T3 transport is significantly impaired in MCT8-deficient primary cortical neurons. Systematic analysis of candidate thyroid hormone transporters in primary cortical neurons identified several other transporters that may be involved in compensation of the neurological phenotype almost absent in mice. Since some organs show either a hypothyroid, normal, or hyperthyroid phenotype, we speculate that every organ and tissue may be equipped with different thyroid hormone transporters and their differential response may depend on whether MCT8 is limiting T3 transport or not.
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P709

Cinacalcet reduces serum calcium in intractable primary hyperparathyroidism (PHPT)

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Patients with persistent PHPT after parathyroidectomy (PTX) or contraindications for PTX often require chronic treatment for hypercalcemia, representing an unmet medical need. In an open-label, single-arm study in the US and EU, 17 patients underwent a variable length titration phase (maximum 16 weeks) with a maintenance phase (MP) of up to 3 years. Patients were eligible if they had (1) persistent PHPT following PTX or were considered contraindicated for PTX, and (2) serum calcium (sCa) >3.1 mmol/l (12.4 mg/dl). Possible dose increases occurred every 2 weeks during a titration phase until sCa ≤ 2.5 mmol/l (10.0 mg/dl), or the patient reached the highest dose (90 mg qid) allowed, or adverse events (AEs) precluded further dose increases. The primary endpoint of sCa reduction by ≥ 0.25 mmol/l (1 mg/dl) at the end of titration (EOT) was reached by 15 patients, while 9 reached the secondary endpoint of sCa reduction to ≤ 2.6 mmol/l (10.3 mg/dl) at the EOT. Mean values (SE) for sCa and iPTH showed a decrease in sCa and a rise in iPTH. However, median iPTH (Q1, Q3) decreased from 29.3 pmol/l (14.9, 33.0) at baseline to 19.0 pmol/l (14.4, 32.4) at EOT. Three patients were treated for up to 72 weeks in the maintenance phase, at which point mean (SE) sCa was 2.5 (0.23) mmol/l and mean (SE) iPTH was 11.9 (4.5) pmol/l. The most frequent treatment-related AEs were nausea, vomiting, and paresthesia. Only 1 patient had a treatment-related adverse event that led to withdrawal from the study.

	Baseline	End of titration
Mean sCa (S.E.M.)	3.2 (0.05) mmol/l, $N=17$	2.6 (0.08) mmol/l, $N=17$
Mean iPTH (S.E.M.)	26.7 (2.9) pmol/l, $N=16$	43.5 (17.4) pmol/l, $N=15$

In conclusion, cinacalcet addresses an unmet need in patients with PHPT and hypercalcemia for which no approved therapy exists.

P710

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P711

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P712

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P713

Subclinical hyperthyroidism, a retrospective study for 11 years

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Our hospital has a referent population of 50 000 people in a rural area. We have made a retrospective study about ambulatory outpatients who had been diagnosed with subclinical hyperthyroidism.
Objectives
To describe and analyze the characteristics of these patients, the treatment they received, the relationship beween TSH and goiter, complementary image tests and evolution.
Material and methods
This is a descriptive study about patients registered as subclinical hyperthyroidism during the period from 1995 to 2006: sex, BP, BMI, CF, age at diagnosis, symptoms, TSH and T4L levels, antithyroidal antibodies, sonography and scintingrathy, treatment and evolution. Patients taking thyroid hormone, with subcute thyroiditis, *post-partum* thyroididtis or administration of iodine products were excluded from the study.
Results
$N=57$, m age 64, >65 years 50.87%. About 85.5% women. m BMI 27.6, 508% SBP$\geq$140, 52.6%, DBP$=>$80 m CF 82.6, TSH$<$0.1, 73.7% (40% of them had multinodular goiter, 16% uninodular; rate no significant) TSH$=>$0.1, 26.3%. Antithyroglobulyn antibodies 8.7%, TPO 7%. Multinodular goiter 36.7%, Nodular 14.28%. Heterogeneus 37.8% Sonography 98.24%, Scintigraphy 45.61%. About 52.8% suffered from symptoms. We had registered 21.42% patients with HBP, 10.52% osteoporosis, 8.7% arrhythmia, all of them had TSH$<$0.1. Initial dose of methimazole between 5 and 30 mg. All patients taking 20–30 mg had TSH$<$0.1. Radioiodine treatment 8.77%. A patient undertook surgery. About 31% stopped treatment. The rest maintained the treatment but with a lower dose. Exitus 4. Follow-up by their GP 8, didn't come back 4. Normofuntion at one year: 35 patients, 16% without treatment. Mean follow-up time, 3.6 years.
Conclusion
The majority of patients were women. About 42.1% aged >65. About 80.7% had goiter. About 52.6% had symptoms. When lowering medication TSH disminishes and treatment is manteined at a low dose. TSH levels aren't associated with type of goiter. All patients taking dose$\geq$20 mg had TSH$<$0.1.

P714

Giant subcutaneous hemorrhage mimicking anaplastic thyroid carcinoma

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A 63-year-old woman was referred by her cardiologist because of rapid growth of an anterior cervical mass. The patient had a history of severe vascular atherosclerotic disease, requiring multiple prosthetic vascular surgery, among which thoracic aorta surgery, atrial fibrillation requiring coumadin and chronic respiratory failure. On admission, the patient presented with a 7 cm firm mass, regularly growing for 2 months, adherent to the cervical skin, highly suggestive of thyroid anaplastic carcinoma. Immediately performed, a CT scan demonstrated an anterior cervical lesion, possibly related to an old hematoma. Fine needle biopsy aspiration revealed after thin prep filtration inflammatory cells (macrophages, neutrophil leukocytes and lymphocytes), but no tumor cell. Needle evacuation was poorly successful, and further, regular follow up was decided. No change in the cervical mass volume was observed.
Discussion
A firm rapidly and regularly growing anterior cervical swelling is highly suggestive of anaplastic thyroid carcinoma. Lymphomas and Riedel's thyroiditis may also be evoked. By contrast, intrathyroidal hemorrhage is usually acute and painful. Hematomas of the thyroid region (except after thyroidectomy) are rather unusual. In our case, it was induced by venous flow changes as a consequence of surgical ligation of left innominate vein following aortic replacement, leading to highly increased subcutaneous straining of turgescent collateral veins. Blood leakage was probably enhanced by antivitamin K treatment (especially when irregularly controlled and excessive) and an initial muscular effort (carrying a sofa).
Conclusion
Regularly growing cervical hematomas may occur months after aortic surgery, due to venous straining rearrangement and enhanced by anticoagulation, and therefore clinically mimic anaplastic thyroid carcinoma.

P715

Genetic predisposition for goiters analysed by a case control study

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Background
Iodine deficiency is the most important exogenous factor for the development of goiters and thyroid nodules. In addition, family and twin studies as well as linkage analyses and a genome-wide scan in 18 euthyroid goiter families suggest a genetic predisposition for euthyroid goiters. However, data about the inheritance of goiters are still contradictory. Therefore, we investigated goiter predisposition by a matched case control study.
Patients and methods
Three hundred and seventy-six patients providing written consent were included in the study. All of them were anti-TPO negative. We matched 188 patients with euthyroid or subclinically hyperthyroid goiter (TSH 4.20–0.05 mU/l) with 188 euthyroid controls without thyroid enlargement for age and gender. Family history of the patients was recorded using a standardised questionaire and thyroid ultrasound was performed for patients with goiter and controls.
Results
About 50.5% of patients with goiters showed a positive family history for goiter. In contrast, only 25% of patients with normal thyroids had a positive family history for goiter ($P<0.001$; OR$=3.1$). Patients with goiters had a significantly higher proportion of parents ($P<0.001$; OR$=3.6$) or siblings ($P=0.004$; OR$=2.5$) with goiters. Children of parents (mother and/or father) with goiters showed a 2.7-fold increased risk for goiter development and had a goiter prevalence of 73.3%. The primary diagnosis of goiter was made 10 years earlier in patients with a positive family history as compared to goitrous patients with a negative family history.
Conclusion
The significantly higher rate of positive family histories in patients with goiters as compared to the matched control patients as well as the increased goiter prevalence in children of parents with goiters indicate the importance of genetic factors in goiter development.
Additionally, the earlier onset of thyroid enlargement in case of a positive family history for goiter further supports a genetic predisposition in the aetiology of goiters.

P716

Effects on bone mineral density of supraphysiological levothyroxine doses in patients with differentiated thyroid carcinoma

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Introduction
The treatment of differentiated thyroid carcinoma exposes most patients to chronic supraphysiological doses of thyroid hormone which seems to have several negative effects for the patient. The goal of the present study was to assess the consequences of these doses on the bone mineral density (BMD).
Patients and methods
We performed a cross-sectional study on 64 female patients followed up between 1972 and 2007. We registered the levothyroxine doses over the last 3 years and the anthropometric parameters. All patients were tested for their hormonal situation and underwent a BMD study on hip and lumbar spine by DXA. For the statistic analysis, we performed the corresponding parametric and nonparametric tests to compare means and establish correlations.
Results
Out of 64 patients, 71.4% ($n=45$) were menopausal and 28.6% ($n=19$) were premenopausal. The time of evolution was higher in the menopausal patients (15.9 vs 8.8 years, $P<0,05$) and the levothyroxine dose was higher in the premenopausal patients (2.45 vs 2.15 μg/kg, $P<0,05$). According to the levothyroxine dose, those patients receiving over 2.2 μg/kg or 100 μg/m^2 showed a decrease in their T and Z Scores, compared with those who were receiving a lower dose. The results were significant in the premenopausal women, in both areas, whereas in the menopausal ones, the significant results were those in the lumbar spine. The menopausal patients who had received a dose over 100 μg/m^2 had osteopenia in the lumbar spine (Spine T Score -1.54).
The dose of levothyroxine in premenopausical patients had a negative and significant correlation with hip T Score ($r=-0.692$) and spine T Score ($r=-0.470$).

Conclusion
In our study, those patients receiving higher doses of levothyroxine showed a decrease in the BMD parameters. In the menopausal patients, this decrease resulted in osteopenia in the lumbar spine.

P717

Autoantibodies to thyroid peroxidase and hypothyroidism in patients with type 1 diabetes

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Objective
Genetic susceptibility to autoantibody formation in association with autoimmune thyroid disease and type 1 diabetes mellitus has been described with varying frequencies. We have, therefore, investigated the prevalence of antithyroid peroxidase (anti-TPO), overt and subclinical hypothyroidism in type 1 diabetic patients.
Methods
Sixty-five subjects with type 1 diabetes mellitus and 65 unrelated normal controls were recruited for the detection of anti-TPO and Thyroid-stimulating hormone (TSH). Written informed consent was obtained after the procedure had been fully explained.
Results
Among 65 type 1 diabetic patients, 18 (27.7%) were positive for anti-TPO and 18 (27.7%) had abnormal serum TSH level. Among these patients 11 (16.0%) had overt hypothyroidism and 7 (10.8%) had subclinical hypothyroidism. Compared with those without thyroid autoimmunity, there was a female preponderance for the type 1 diabetic patients with thyroid autoimmunity (female:male, 24:23 vs 11:7 respectively). Patients with thyroid antibodies were older, had a longer duration of diabetes (17.6 ± 9.3 vs 10.8 ± 7.8 years), and developed diabetes later in life than those without antibodies. The mean anti-TPO levels were higher in patients with overt hypothyroidism (238.18 ± 223.69 U/ml) than in patients with subclinical hypothyroidism (36.38 ± 22.46 U/ml). Among 11 patients with overt hypothyroidism 9 (81.1%) had abnormal anti-TPO levels, whereas positive anti-TPO were detected in 57% of patients (4:7) with subclinical hypothyroidism.
Conclusion
The presence of anti-TPO in 27.7% of our type 1 diabetic patients confirmed the strong association of autoimmune thyroid disease and type 1 diabetes mellitus. For early detection of autoimmune thyroid disease in patients with type 1 diabetes mellitus, measurement of anti-TPO and TSH preferably at type 1 diabetes mellitus onset is recommended.

P718

Hypothyroidism after transarterial chemoembolization for hepatocellular carcinoma

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Transarterial chemoembolization (TACE) is widely used for treatment of hepatocellular carcinoma (HCC). During this procedure approximately 20 g of iodide is applied by radiopaque material. There is usually high awareness regarding hyperthyroidism in association with contrast enhanced imaging studies and TACE. To date, however, there are no reports regarding the frequency of hypothyroidism after this procedure, although high doses of iodide can also inhibit the synthesis of thyroid hormones (Wolff Chaikoff effect).
We retrospectively evaluated a cohort of patients with HCC treated by TACE at our center from 1997 to 2007. Out of 107 patients with histologically proven HCC, 65 had TSH measured before and after at least one TACE. Treatment was performed one to seven times. Twelve out of 65 patients (18.5%) had a suppressed TSH. Two were treated for hyperthyroidism, 10 had subclinical hyperthyroidism, either before or after TACE.
Surprisingly, 8 out of 65 patients (12.3%) developed an elevated TSH after the TACE (mean TSH 19.96 μU/ml, range 4.59–76.66). Two of them displayed transient hypothyroidism with normalization of TSH within months. The course of hypothyroidism in the other 6 patients was lost to follow-up. TPO antibodies were only measured in two patients, one was negative and the other was antibody positive.
The high prevalence of hypothyroidism after TACE should lead to enhanced clinical suspicion of this disorder in these patients.

P719

Alterations of the circulating selectin, intercellular adhesion molecule-1, and vascular cell adhesion molecule-1 in patients with non-immune nodular thyroid disease

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Cytokines, including adhesion molecules, are a family of protein mediators that are important in transducing information between various cell types, were non-immune cells may also be important sources of certain cytokines.
Aim
Aim of this study was to evaluate of soluble intercellular adhesion molecule-1 (sICAM-1), soluble vascular cell adhesion molecule-1 (sVCAM-1), and soluble E-selectin (sE-selectin) in patients with non-immune nodular thyroid disease.
Materials and methods
We formed two study groups: 32 patients with non-toxic, non-immune nodular thyroid disease (P) and 32 healthy subjects were selected as controls (C). The histological examination revealed follicular adenomas in all of the cases. All patients were without autoimmune diseases, cancer and coronary heart disease. The study groups were matched for age, sex, and body mass index. sICAM-1, sVCAM-1 and sE-selectin were measured by xMAP technology (Luminex-200 analyzer).
Results
sICAM-1, sVCAM-1 and sE-selectin levels were statistically significantly elevated in patient group compared to healthy subjects (P versus C, $P<0.05$).
Conclusion
Our findings show that patients with non-immune nodular thyroid disease have significantly elevated sICAM-1, sVCAM-1 and sE-selectin levels.

P720

Level of von Willebrand factor in thyrotoxicosis of a various genesis

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In several studies, the state of the endothelial function is analyzed in clinical thyrotoxicosis. Increased level of von Willebrand factor is detected. Many events of association of trombosis at these patients with high level of VWF are described. An association between autoimmune thyroid disease and pulmonary arterial hypertension (PAH) has been reported too. We have studied factors which influence on level of VWF at patients with a clinical thyrotoxicosis of a various genesis. The present study includes 74 normotensive patients with a thyrotoxicosis of Graves' disease (GD) and 15 patients with a thyrotoxicosis of not immune genesis (TNIG) (toxic nodular goitre) without any CVD. The level of von Willebrand factor was mesured by the immunoturbidimetric method (the normal range – 50–160%). The patients were examined echocardiography by standard method. The mean level of VWF had been increased ($181.6 \pm 13.57\%$) before the therapy. The mean level of VWF has been increased ($192.95 \pm 13.74\%$) in group of patients with GD in comparison with group TNIG ($120.5 \pm 11.29\%$) ($P<0.01$). Correlation analysis detects the relationship between VWF and a level of thyroid hormones: fT3 ($r=0.38$, $P<0.01$), fT4 ($r=0.27$, $P<0.05$) and an antibody to TSH ($r=0.26$, $P<0.05$) and an antibody to TPO ($r=0.32$, $P<0.01$). From EchoCG parameters the moderate correlation with pressure in pulmonal artery (PPA) ($r=0.27$, $P<0.05$) is noted and very strong relationship is detected between level of VWF and PPA at the dynamic control over year ($r=0.86$, $P<0.001$). After treatment level of VWF has been decreased ($97.8 \pm 6.85\%$) in comparison with first examination ($P<0.01$). These results demonstrated, firstly, that rising of a level of a VWF is associated with an autoimmune genesis of a thyrotoxicosis, secondly, that these changes can be a predictor/marker of a PAH. Immune system dysfunction may underlie this association.

P721

Clinical impact of positron emission tomography/computed tomography in the follow-up of well differentiated thyroid carcinoma with elevated anti-thyroglobuline auto-antibodies

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Aim
Assessment of the clinical impact of a positron emission tomography/computed tomography (PET/CT) with FDG in the management of differentiated thyroid cancer (DTC) in patients with increased or positive thyroglobulin autoantibodies (AbTg).
Methodology
Retrospective study involving 15 patients seen in the follow-up with confirmed DTC primarily treated with total thyroidectomy and Iodine 131. Patients presenting with increased AbTg and a negative or non informative conventional evaluation were included. Inclusion was independent of presence or not of metastatic lymph nodes at time of diagnosis. The results of the PET/CT were correlated with histology and/or clinical follow up. The clinical impact was determined on a change of intention to treat, which was decided upon in multidisciplinary meetings, based on the PET/CT result.
Results
We observed in 14 out of 15 eligible patients: 10 true positive examinations (confirmed by histology in 7 patients and clinical follow up in 3) 7 patients with cervical uptake, 1 cervical and mediastinal uptake, 1 cervical and lung uptake and 1 liver uptake, 4 true negative examinations with negative follow up of on the average 22 months (18–36 months) and no false positive or false negative results. The intention to treat was modified in 73.3% (11/15 patients). The variation in the values of the AbTg of the true positive and the true negative group was similar and did not allow for a threshold value.
Conclusion
PET/CT with FDG seems to be very useful in the therapeutic management of recurrence of DTC in the case of increased or positive AbTg, in particular for the patients with N1 at the initial staging. Further studies are suggested to confirm the very promising negative predictive value and specificity of this study in a larger number of patients with longer follow up.

P722

Thyroid hormone receptor beta gene mutation (P453A) in a family producing resistance to thyroid hormone

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Background
Resistance to thyroid hormone (RTH) is a dominantly inherited syndrome characterized by decreased responsiveness of target tissues to thyroid hormone. Two members of a Turkish family, a mother and son, had thyroid function tests suggestive of resistance to thyroid hormone (RTH).
Methods
The clinical presentation was, however, different. The mother (proposita) had palpitation, weakness, tiredness, nervousness, dry mouth and was misdiagnosed as having multinodular toxic goiter which was treated with antithyroid drugs and partial thyroidectomy. Her younger son had attention deficit hyperactivity disorder and primary encopresis, but normal intellectual quotient. Both had elevated serum iodothyronine levels with nonsuppressed thyrotropin.
Results
A mutation in one allele of the thyroid hormone receptor beta gene (P453A) was identified, providing a genetic confirmation for the diagnosis of RTH.
Conclusion
Mutational analysis of the TRß gene allows definitive diagnosis of RTH, potentially avoiding the need for protracted and expensive pituitary function testing.

P723

Prevalence of antibodies to thyroid peroxidase (TPO) and postpartum thyroiditis (PPT) in a cohort of 1700 pregnant women with gestational diabetes mellitus (GDM)

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The prevalence of antithyroid autoantibodies in pregnant women ranges between 6 and 10%, like in the general population. PPT, a syndrome of transient or permanent thyroid dysfunction occurring in the first year after delivery, is associated with the presence of TPO in gestation. The prevalence varies from 5 to 10%, with higher frequency in patients with DM1. The aim of this study is to estimate TPO frequency during gestation and its relationship with PPT in a group of women with GDM.
Methods
GDM was diagnosed in a cohort of 1700 pregnant women (mean age 32.7 ± 6.5 years; mean gestational week: 26.2 ± 5.1), excluded patients with previous thyroid dysfunction. In 1053 patients (60.3%) a follow-up of thyroid function after delivery was performed (3.5 ± 3.3 months).
Results
The 22.7% of the patients were TPO positive (>11.9 U/ml). A clear association between the presence of TPO and recurrent pregnancy loss (≥ 3 miscarriages) was found (RR: 2.41; CI 95%: 1.17–4.97). The prevalence of hypothyroxinemia in the cohort was 5.1%, regardless of autoantibodies levels. Positive TPO frequency postpartum was 39.9%. Women with positive TPO during pregnancy had a greater risk to maintain a positive titer in postpartum (RR: 2.63; CI 95%: 2.30–3.01). The PPT prevalence in pregnant with positive TPO during gestation was higher (RR: 3.76; CI95%: 2.61–5.42).
Conclusions
Considering the high prevalence of positive TPO in women with GDM and the increased risk of developing PPT in this group, a screening of thyroid function during pregnancy and a postpartum follow-up is recommended in this women. There is a significant association between the presence of thyroid autoimmunity and a higher miscarriage rate.

P724

Subclinical hyperthyroidism and insulin resistance in women with simple goiter

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Introduction
Thyrotoxicosis has been shown to cause deterioration of glucose levels in patients with DM 2 while return to euthyroidism results in better glycaemic control. Studies in normal subjects suggest that increased thyroid hormones may aggravate insulin resistance and decrease pancreatic secretion of insulin but the exact underlying mechanisms have not been completely elucidated. The purpose of this study was to investigate if this also occurs in subclinical hyperthyroidism as in the case of thyroxin suppression therapy in women with simple goiter.
Patients and methods
We prospectively studied 32 premenopausal women with simple goiter: 16 normal cycling women (Group A) and 16 women with polycystic ovary syndrome (PCOS) (Group B) matched for age and weight. We measured T3, T4, TSH, Insulin, C-peptide and glucose levels, before and 3 months after suppression treatment with thyroxin 2 μg /kg per day. For the assessment of insulin resistance HOMA-IR was calculated. Comparisons between groups were made with Wilcoxon matched pairs test.
Results
As expected, there was a significant increase in T4 and a significant decrease in TSH in both groups ($P<0.0001$). In Group A, glucose and C-peptide levels and HOMA-IR were significantly decreased after thyroxin therapy while insulin levels remained unchanged (glucose 94.4 ± 3.0 vs 80.8 ± 2.4 mg/dl $P<0.004$, C-peptide 2.92 ± 0.27 vs 1.76 ± 0.14 ng/dl, $P<0.0004$, HOMA-IR 4.1 ± 0.27 vs 3.5 ± 0.1, $P<0.004$). In Group B, only insulin levels and HOMA-IR were significantly reduced (insulin 20.1 ± 10.6 vs 11.1 ± 4.2 μIU/ml, $P<0.02$,

HOMA-IR 4.8 ± 1.2 vs 2.5 ± 0.25, $P < 0.05$) after thyroxin therapy. No significant change was noted in body weight as well in T3 levels during the study.
Conclusions
In contrast to primary hyperthyroidism, suppression thyroxin therapy in women with simple goiter as well as women with simple goiter and PCOS results in reduction of insulin resistance and better glucose tolerance.

P725

Treatment with thyroxine reduces thyroid volume in euthyroid children with Hashimoto thyroiditis

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Introduction
There is no concensus whether euthyroid children with Hashimoto's thyroiditis (HT) need treatment with thyroxine.
Aim of the study
To assess whether thyroxine influences goitre progression (calculated thyroid volume on U/S scan) in euthyroid children with HT.
Subjects and methods
We studied 50 euthyroid children with HT for a 2-year period. Children with a multinodular goitre were not included in the study. Twenty-five children (17 girls and 8 boys, median age (IQR) 12.1 (11.1–13.4) years) were randomised to receive thyroxine and 25 children (20 girls and 5 boys, age 12.2 years (11.1–13.0)) did not receive treatment and were followed-up. There were no significant differences in sex, age, height, height SDS, weight, weight SDS, BMI, BMI SDS, thyroid volume (7.7 (6.6–9.1) ml and 7.3 (6.2– 8.4) ml, respectively and thyroid volume SD (1.1 (0.7–1.5) and 0.9 (0.4–1.4)) between the two groups.
Results
Following one year there was no significant difference in the thyroid volume (7.1 (5.8–10.2) and 9.0 (7.9–10.6) ml, respectively, $P = 0.128$) and thyroid volume S.D. (1.0 (0.0–1.4) and 1.7 (0.8–2.0), $P = 0.075$) between the treated and the non treated group. Three children of the non treated group who developed hypothyroidism and were treated were excluded from further analysis. Following 2 years, the children on thyroxine had significantly smaller thyroid volume (7.6 (6.3–9.2) vs 10.6 (8.2–12.1) ml, $P = 0.016$) and thyroid volume S.D. (0.6 (0.3–1.0) vs 2.0 (1.1–2.3), $P = 0.001$) compared to the children that did not receive treatment. When each group was studied separately and comparisons within groups were made, there was no significant difference in the thyroid volume nor in the thyroid volume S.D. before and after 1 year of treatment, however thyroid volume S.D. was significantly lower two years following treatment compared to the thyroid volume before treatment ($P = 0.002$, Wilcoxon signed rank test). In the non treated group, thyroid volume and thyroid volume S.D. increased after the 1st year ($P < 0.0001$ and $P = 0.007$ respectively) and 2nd year ($P = 0.001$ and $P = 0.016$) of follow-up.
Conclusion
Treatment with thyroxine is beneficial for the further progression of goitre because it reduces thyroid volume significantly in euthyroid children with HT.

P726

Surgical management of amiodarone-associated thyrotoxicosis

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Introduction
Amiodarone is an excellent antiarrhythmic with a high use although it isn't free from complications. Most of patients stay euthyroid (80%), inducing thyroid dysfunction in the remaining 20% (thyrotoxicosis, 1.5–3%).
Objective
We analyze amiodarona-associated hyperthyroidism surgical management according to the associated pathology and patients clinical evolution.
Subjects and methods
We report four patients with amiodarona-associated hyperthyroidism occurred between September 05 and March 07, two women and two men from 44 to 74. Patient 1: chronic bronchitis, paroxysmal atrial fibrillation (PAF). Treatment: antithyroids and corticosteroids. Toxic hepatitis induced by metimazol. 1 month of hyperthyroidism. T4L 6.1 ng/dl. Patient 2: Arterial hypertension, FAP. Treatment: antithyroids and corticosteroids. Four months of hyperthyroidism. T4L 7.5. Patient 3: HIV, chronic hepatitis, chronic renal failure, arterial hypertension, moderate aortic stenosis, PAF. Treatment: antithyroid and corticosteroids. Two months of hyperthyroidism. T4L 6.5. Patient 4: vascular encephalopathy, heart failure (double mitral valve, tricuspid failure valve, pulmonary hypertension), PAF. Treatment: antithyroids, corticoids and potassium perchlorate. Two months of hyperthyroidism. T4L 7.7.
Results
Patient 1: thyroidectomy without peri-postoperative complications. Median hospital stay 2 days. TSH 0.1 mcU/ml and T4L 1.7 ng/dl. Patient 2: thyroidectomy without peri-postoperative complications. Median hospital stay 2 days. TSH 0.1. T4L 1.6. Patient 3: not surgical indication (severe plaquetopenia). TSH 4.3. T4L 1.5. Patient 4: not surgical indication (comorbidities). TSH 5.8. T4L 1.1.
Conclusions
1. Although comorbidities involve a high cardiovascular surgery risk, total thyroidectomy doesn't mean more difficulties and a higher rate of complications shouldn't be expected.
2. Surgical treatment arises when it is not possible to discontinue treatment with amiodarone, when complications appears from the use of antithyroids and when is necessary an early symptomatic control.
3. There is a need for an assessment of each patient in order to establish a safer therapeutic approach.

P727

Hashimoto's thyreoiditis is associated with an increased occurrence of deficits in attention testing compared to patients with other thyroid illnesses

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Objectives
Experimental and clinical data point to an involvement of the central nervous system in Hashimoto's thyroiditis (HT) independent of thyroid dysfunction. The neuropsychological function of patients with HT has not been systematically evaluated yet.
Design
In the present prospective study, neuropsychological testing was performed in 26 euthyroid patients with HT compared to 25 euthyroid patients with hormonal treatment for goitre or after thyroid surgery.
Results
Investigating executive function, cognitive flexibility, attention, figural and verbal memory as well as acoustic working memory with established neuropsychological tests no significant differences between the two groups could be detected. However, comparing the number of patients with conspicuous test results we found significantly more patients with a performance below the cut-off point in the d2 attention test regarding carefulness (GZ-F, HT versus control group: 12 vs 5, $P < 0.05$) and attention (KL, HT versus control group: 12 vs 2, $P < 0.01$) than in the control group. Comparison of the demographic and endocrine data of the HT patients revealed a significantly increased mean value of the anti-thyroid peroxidase antibodies (TPO-Abs) in the group with conspicuous results in the d2 attention test (371.4 ± 187.2 vs 69.3 ± 28.7, $P < 0.05$).
Conclusions
The results of the present study point to subtle cerebral dysfunction in a part of patients with HT even in the euthyroid state. These patients might have an increased risk to develop a neuropsychiatric disease. Disturbances of cerebral thyroid hormone metabolism by the anti-thyroid antibodies or an association with an unknown autoimmune disorder affecting the central nervous system could possibly explain the findings.

P728

The impact of changed body image on social relations and interpersonal behavior for patients with thyroid associated orbitopathy

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Objective
Altered appearance and changed visual function characterize thyroid associated orbitopathy (TAO). TAO affects face and eyes and influences social function. It is well documented that thyroid disease influences the patient's quality of life negatively and TAO aggravates the situation. Recently, attention has been directed towards the impact of the patient's body image and studies show how body image dissatisfaction is a significant psychosocial complication. Although TAO is a disfiguring disease the impact of change in body image on social function has not been investigated using qualitative methods. This study explores how patients with TAO experience the impact of a changed body image on their social relations and interpersonal behavior.
Methods
An ethnographic approach using interviews and participant observation. Thirteen patients diagnosed with moderate to severe TAO and receiving treatment in a thyroid clinic were included. Grounded theory methodology was used to analyse data. The local ethics committee approved the study.
Results
The participants' ability to maintain their social function was connected with changed body image. 'Eyes out of control' was the dominating experience, including 4 dimensions; deviating appearance, deviating visual function, deviating visual sensibility and deviating disease. TAO made it difficult to make eye contact with other people and use the eyes for communication. The participants witnessed how people stared at them, acted unpleasantly, avoided them, and misunderstood their facial expression. TAO made the participants change their social function with regard to reduced contact with others and everyday activities such as shopping, reading, driving and working.
Conclusion
TAO is a challenging disease, hard to treat and unpredictable, making it difficult for patients and others to understand the meaning of TAO. The findings of this study contribute to clarification of essential elements of living with TAO and development of guidelines for supporting and informing patients with TAO.

P729

Valuation of iodine deficiency in Western Andalusia by means of the neonatal screening of congenital hypothyroidism

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Introduction
The accomplishment of screening neonatal congenital hypothyroidism allows the use of the determination of TSH in new born (NB) like indirect marker of the iodine ingestion of the pregnant woman and the nutritional iodine deficiency in the population. In populations with a sufficient iodine ingestion, the proportion of children with TSH superior to 5 U/ml is smaller of 3%.
Primary target
To make the iodine deficiency map in the studied area (western Andalusia).
Method
The NB studied were selected by measuring TSH levels in heel dry blood from the provinces of Cadiz, Huelva and Seville during years 2000–2004 (188, 616 RN). The percentage will take shelter of NB that in each population has a value of TSH$>$5 uU/ml., indicative according to the WHO/UNICEF/ICCIDD of iodine deficiency. It was used only samples of heel with a stagger between the birth and extraction of $>$48 h to avoid the slant of confusion of transitory physiological TSH elevation.
Results
Percentage of RN with TSH$>$5 uU/ml (we frequently presented/displayed in the single summary the ZBS$>$3%)
PROV. HUELVA 4.11%
DISTRICT COUNTY COUNTRYSIDE 2.72%
DIST. HUELVA COST 4.55%
DIST. MOUNTAIN RANGE OF CENTRAL HUELVA ANDEVALO 12.50%
PROV. SEVILLE 1.06%
DIST.ALJARAFE 1.19%
DIST SEVILLE CITY 1.20%
DIST EAST SEVILLE AREA OSUNA 0.74%
DIST NORTH SEVILLE 0.83%
DIST SOUTH SEVILLE 0.94%
PROV. CADIZ 2.48%
DIST BAY OF CADIZ JANDA 2.10%
DIST FIELD OF GIBRALTAR 1.76%
DIST SHERRY COSTA NE 3.56%
DIST MOUNTAIN RANGE OF CADIZ 2.90%
CEUTA 1.85%
MELILLA 8.94%
Conclusions
Zones of iodine deficiency still exist in western Andalusia which means that a sanitary intervention is necessary to prevent the upheavals associated to this situation in order to its clinical importance.

P730

A poly A(−) RNA transcript (Heg) in human blood mononuclear cells is inversely related to TSH receptor autoantibodies in patients with untreated Graves' disease: gene expression of CD14 and inhibition of Cdk1

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Objective
We studied a previously uncharacterized poly A(−) RNA transcript (designated Heg) in blood mononuclear cells and its relationship to TSH receptor autoantibodies and to CD14, Nucks, Cdk1, GCRα, NF-κB and CK2 mRNA.
Subjects and methods
The main study groups were a) normal subjects b) patients with early and untreated Graves' disease and c) patients with Graves' disease studied after treatment. Quantification of mRNA was done by RT-PCR-HPLC. The protocol study was approved by the local Ethics Committee.
Results
The sequence of the transcript was localized to a clone from the HUGO project. The 3′ end of the Heg gene overlaps the 3′ end of the Nucks gene. In 18 normal subjects, mean basal levels of specific RNA/mRNA were 0.15 ± 0.01 (mean $\pm$ S.E.M.), 1.3 ± 0.07 and 30 ± 3 amol/μg DNA for Heg, Nucks and CD14, respectively. In 17 patients with untreated Graves' disease, concentrations of TSH receptor autoantibodies were negatively correlated to Heg RNA amol/μg DNA ($P<0.001$) and positively correlated to Cdk1 mRNA zmol/μg DNA ($P<0.002$) ($r=0.83$). In the combined group of treated patients with Graves' disease and normal subjects CD14 was negatively correlated to Heg RNA amol/μg DNA ($P<0.001$). Nucks and CK2 mRNA were closely correlated ($P<0.001$). Inhibition of Cdk1 by a cyclin-dependent kinase inhibitor, Roscovitine, decreased CD14 mRNA markedly ($P<0.001$). Heg and Nucks siRNA elicited an immune reaction.
Conclusions
TSH receptor autoantibodies may be regulated by nuclear kinase activity (Cdk1) and the degree of apoptosis (Heg) in mononuclear cells. Drugs which inhibit Cdk1 may be of interest in the treatment of autoimmune disease.

P731

Rate of thyroid cancer in a cohort of thyrotoxic patients treated with radioactive iodine

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Radioactive iodine treatment for thyrotoxicosis had been introduced since 1940(s). The association of thyroid cancer and radioactive iodine (RAI^{131}) treatment for thyrotoxicosis is rare. Rate of thyroid cancer in Jordan is 3 per 100 000 (according to Jordan National Registry).The latest population is estimated to be 5.6 million in 2006.
Aim
To look for rate of thyroid cancer in a cohort of patients who received RAI^{131} and to report the index cases' characteristics and management.
Patients and methods
A cohort of 227 patients who received RAI^{131} over period of 10 years (1997–2007) for different causes of thyrotoxicosis were followed up by physical exam and serial thyroid function tests.
Results
We found two cases of thyroid cancer during this follow up period giving a prevalence of 0.88% of cases who received RAI^{131}. Relative risk$=$39.32 (95% confidence limits for RR 9.88$>$RR$<$156.46) $P=0.0012$.
First case is a 75-year-old female patient who was diagnosed to have thyrotoxicosis due to toxic multinodular goiter in 1990 who received 25 mCi RAI^{131} in June 1998. She became clinically and biochemically euthyroid. In May 2006, she noticed progressive neck swelling causing compressive symptoms and

hoarseness of voice. Thyroid FNA revealed medullary versus anaplastic cancer. Serum calcitonin level was elevated at 11.3 pg/ml (normal value: 0–5) favouring medullary thyroid carcinoma. An emergency surgery to relieve obstruction was attempted, but unfortunately she succumbed immediately postoperatively. Histopathological diagnosis confirmed medullary thyroid carcinoma with anaplastic changes and positive calcitonin stain. Second case is 65-year-old lady, known case of bronchial asthma and toxic multinodular goitre. She received 15 Mci RAI^{131} in 2003 and became euthyroid within 6 months. In April 2007, she presented with neck discomfort and sensation of suffocation. Thyroid FNA biopsy revealed papillary thyroid cancer. The diagnosis was confirmed histologicaly after total thyroidectomy. She is due for ablative RxI^{131}.
Conclusion
The prevalence of thyroid cancer post RAI^{131} is 0.88%. Despite no direct cause effect relationship between RAI^{131} and thyroid cancer could be established in these cases nevertheless they highlight the importance of lifelong surveillance of patients who receive RAI^{131}.

P732

Correlations between lipids and thyroid hormones in primary hypothyroidism

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The aim of the study was to assess the influence of thyroid hormones on lipid profiles in primary hypothyroidism, in order to determine the most influential one for the clinical follow-up. Study group included 15 subjects (3 male, 12 female, age 46 ± 13 years/mean $\pm$ S.D.), and total and free T4, total and free T3 and TSH, total, HDL and LDL cholesterol, triglycerides, ApoA, ApoB and Lp(a) were determined. The diagnosis of primary hypothyroidism was based on increased TSH and decreased FT4 concentrations. We found that only FT3 was inversely correlated with total cholesterol, triglycerides and ApoB concentrations. Other assessed hormones were not significantly correlated with any of the measured parameters. Therefore, FT3 should be assessed in patients with patients with primary hypothyroidism and lipid disorders.

P733

The role of pentagastrin stimulated calcitonin in nodular thyroid disease: will we be able to minimize false positive results?

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An increase in basal and Pentagastrin (Pg) stimulated calcitonin (CT) concentration is a specific feature of medullary thyroid carcinoma (MTC). Routine basal CT measurement in nodular thyroid diseases is controversial. Pg stimulation is expected to increase the basal CT specificity in MTC screening, although false positive responses in patients without MTC have been reported.
Aim
To report the diagnostic accuracy of Pg-stimulated CT in surgically treated patients with nodular thyroid diseases and basal CT in the high-normal range or slightly supranormal (<42 pg/ml).
Patients and methods
We studied 12 pts (11 M, 1 F, aged 55.7 ± 24.1), without evidence of MTC at pathological examination (nonMTC-group) and 9 pts (1 M, 8 F, aged 37.1 ± 26.3) with pathologically proven MTC (hereditary in 6 cases) (MTC-group). Tumor size in MTC-group was comparable in hereditary and non-hereditary cases ($P = 0.129$). Serum CT was measured (Immulite 2000 Calcitonin, Medical Systems, normal values 0–15 pg/ml) in basal condition and 1, 2, 4 and 10 min after i.v. injection of Pg (0.5 mcg/kg). ROC curve was performed by MedCalc-package.
Results
Mean basal and Pg-stimulated CT concentrations were 20.6 ± 8.1 (range 10.2–39) and 119.5 ± 77.7 (range 21–281) in nonMTC-group and 28.6 ± 8.9 (range 13.4–41.5) and 685.7 ± 593 (range 116–1600) in MTC-group. In nonMTC-group, Pg-stimulated CT levels were higher in cases with pathological evidence of C-cell-hyperplasia (CCH) than in cases without CCH (172.3 ± 69.2 vs 66.7 ± 42.5, $P < 0.05$).
At ROC-analysis a Pg-stimulated CT value >193 pg/ml showed the best accuracy in detecting MTC with 77.8% sensitivity, 92.9% specificity; positive and negative likelihood ratios were 87.5 and 84.6%.
Conclusions
On considering the large orverlap of PG-stimulated CT in patients with and without MTC, any cut-off value chosen as a criterium to recommend surgery in patients with nodular thyroid disease remains fallible and possibly bound to change over time.

P734

Resistance to thyroid hormones (RTH): study of a family

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Resistance to thyroid hormones (RHT) is a rare syndrome, with autosomic dominant transmission, due to mutations in thyroid hormones beta-receptor gene. Clinical presentation is variable for the same mutation. This hypothesis must be considered in presence of high levels of thyroid hormones and TSH not suppressed.
The evaluation of a 15-year-old female patient, in 1990, harbouring a thyroid nodule, secondary amenorrhea and visual and auditory impairment showed slightly elevated thyroid hormone levels but normal TSH. She started levothyroxine treatment and resumed menses. She was lost to follow up. Three years later, returned to consultation with a multinodular goiter, high levels of thyroid hormones and high TSH, in spite of levothyroxine treatment. After TRH stimulation and pituitary MRI, resistance to thyroid hormones was diagnosed. Treatment was tried with tri-iodotironine and TRIAC, with slight reduction of TSH. In 2004, a new radiologic evaluation suggested a pituitary microadenoma, fully described with 10 mm in 2006. No other hormonal disorders were detected. A not yet described mutation in exon 9 (G344R) was identified by Professor Beck-Peccoz team. We studied patient's family – mother, two brothers and two sisters (one of them is a non-identical twin sister of our patient). Father was already deceased. We found abnormalities in thyroid hormone levels in both brothers, who also have the mutation. MRI of one of them suggests a pituitary microadenoma.
Conclusions
We describe a family with Resistance to Thyroid Hormones Syndrome due to a new mutation in thyroid hormone receptor beta – G344R. Until now, a pituitary microadenoma was diagnosed in two of the affected patients, which is a rare pathological situation that can occur in the natural history of RTH.

P735

Onset of thyrotoxicosis in patients over 65 years is associated with increased risk of skeletal and heart disease

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It has been shown that aging is associated with 'physiological' alterations in both the hypothalamo–pituitary–thyroid axis and at the peripheral level such as elevation of basal TSH levels, often with normal T_3 and T_4 levels and decreased nuclear binding of thyroid hormones, particularly of T_3. The frequency of nodular goiter and of an altered thyroid status increases with advancing age. The aim of our study was to analyze the clinical and paraclinical features of thyrotoxicosis in patients over 65 years of age, hospitalized at the Clinic of Endocrinology Cluj-

Napoca, Romania. The study was performed on 51 patients (9 men and 42 women) with a mean age of 67.6 ± 0.62 years. Aside from clinical data, thyroid ultrasonography, radionuclide imaging of the thyroid, thyroid hormone levels and other laboratory examinations were performed. Graves'disease was observed in only 10 patients while toxic nodular goiter/adenoma was noticed in 41 patients. The majority of hyperthyroid patients over 65 presented with cardiovascular symptoms (96%), followed by tremor (84%) and weight loss (78%). Of the 51 patients, 8 presented with atrial fibrillation. Nervousness, anxiety and hyperkinetic motor activity were seen in only 40% of patients. The most sensitive laboratory test for the diagnosis of hyperthyroidism was the concentration of basal TSH. Serum alkaline phosphatase concentrations were slightly increased (56.1 ± 4.1 U/l) probably due to an enhanced rate of bone remodeling. Screening for osteoporosis by ultrasonography revealed the presence of the disease in 30% of patients while 60% had osteopenia. In 17 patients, radioiodine therapy or surgical ablation of the gland was decided.
Conclusions
The study suggests that at the time of presentation hyperthyroidism in elderly is associated to a distinct clinical pattern. With regard to the complications of hyperthyroidism, aged patients are more likely to develop cardiovascular and skeletal abnormalities.

P736

Evolution of thyroid autonomy in iodine sufficient environment

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Last 10 years, we are living in iodine sufficient environment. To clarify natural course of thyroid autonomy in this conditions, we investigated 100 consecutive patients in last three months – 93 female and 7 male aged 19–86 years. Included were patients with scans showing thyroid autonomy, and patients with nodular or diffuse goiter with low or suppressed TSH. We followed them from their first visit. Thirty patients were hyperthyroid – one spontaneously cured, 50 had low or suppressed TSH, and 20 had normal both TSH and thyroid hormones. Twenty-five patients presented with mild hyperthyroidism from the very beginning, in 5 patients hyperthyroidisnm developed in next 1–5 years. In two patients, thyroid autonomy developed after cure od autoimmune hyperthyroidism. One patient had papillary thyroid carcinoma and hyperthyroidism in nodular goiter. Considering the fact that hyperthyroid patients have at least one visit in 3–4 months, that those with low TSH, we follow twice a year, and that those with normal hormones, we see once a year, we approximate that 18% of our patients with thyroid autonomy are hyperthyroid.

P737

Role of deiodinases in the thyroid for the maintenance of circulating T3 levels

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Deiodinases (Dio1-3) are selenium-dependent enzymes that catalyze the reductive removal of iodine from iodothyronines. Depending on the substrate and the position of the iodine removed, deiodination can generate T3 from T4 or inactivate T3 and T4. Local expression of Dio can help to adjust the intracellular levels of T3 to the physiological needs of the organ without changing circulating T3 concentrations. It has been suggested that hepatic Dio1 generates circulating T3. However, we have shown that mice with a hepatocyte-specific inactivation of selenoprotein expression do not display altered serum T3, T4, or TSH levels (Streckfuss *et al. Biochem Biophys Res Comm* 2005). In addition, classical gene disruption of Dio1 in the mouse did not reduce circulating T3 values (Schneider *et al. Endocrinology* 2006). Since Dio2-deficient mice are also not deficient in circulating T3 levels, the question for the source of circulating T3 remains open. Since Dios are expressed in the thyroid, we speculated that T3 may also be produced intrathyroidally by 5′-deiodination of T4. In order to investigate this possibility, we have generated mice with a tamoxifen-inducible inactivation of selenoprotein-specific tRNA (gene symbol Trsp) in thyrocytes: Tg-CreER; Trspfl/fl mice. Application of tamoxifen resulted in a significant decrease of thyroidal Dio1 activity providing a model in which to study the role of intrathyroidal deiodinase activity for circulating T3 levels. In pilot experiments, we observed a reduction of plasma T3 levels in these mice. Thus, it may be possible that thyroidal Dio directly contribute to circulating T3 levels. This notion is compatible with reports on physiological variations of the T3/T4 ratio released from the thyroid gland under conditions of stimulation of the TSH receptor by TSH or TSHr autoantibodies or during severe iodine deficiency. Supported by DAAD and DFG grants.

P738

Iodine excretion and prevalence of thyroid dysfunction in the Western Part of Germany: results of the Heinz Nixdorf Recall study

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Objective
While Germany was considered to be an iodine deficient area grade I to II in 1993, iodine supply has increased. With higher iodine uptake, prevalence of autoimmune thyroiditis may increase. Poor data exist on iodine supply and the occurrence of thyroid dysfunction in middle-aged adults in the western part of Germany. The aim of our study was to analyze iodine status, prevalence of TPO-antibodies (TPOab), TSH and fT4 levels in a large population-based sample in the western part of Germany.
Methods
Between 2001 and 2003 serum and casual urine samples were drawn from 4814 participants (50.2% women, 49.8% men, age 45–75 years) of the Heinz Nixdorf Recall study and analyzed by routine laboratory tests. Assay reagents for TSH, fT4 and TPO-Ab were provided by Roche Diagnostics.
Results
We excluded participants with known thyroid disease, thyroid medication (including iodine-containing drugs) and elevated serum creatinine. Of the remaining 3527 persons, TSH and fT4 data were available from 3150 (89.3%). About 96.7% of men and 94.2% of women were euthyroid. Subclinical and overt hyperthyroidism was detected in 1.5 and 0.4% of male and 2.4 and 0.4% of female participants, respectively. About 1.2 and 0.2% of men and 2.2 and 0.8% of women exhibited subclinical and overt hypothyroidism. The median iodine excretion was 128 μg iodine/g creatinine (men: 113 μg/g, women: 152 μg/g; $P < 0.05$). Iodine deficiency (< 100 μg/g) was significantly more frequent in men than in women (37.9 vs 13.0%). High iodine excretion (more than 200 μg/g) was detected in 24.3% of women and 9.0% of men ($P < 0.01$). Elevated TPOab (> 200 U/ml) were also found significantly more frequent in women (5.4%) than in men (1.5%). In men, high TPOab titres were found particularly in those individuals with high iodine excretion (TPOab > 200 U/ml in 2.9% of men with 200–250 μg/g). Interestingly, in women TPOab > 200 U/ml (6.8%) were detected primarily in individuals with an excretion of 100–150 μg/g. About 69.6% (16/23) of women and 41.2% (7/17) of men with TSH levels > 5 mU/l exhibited TPOab > 200 U/ml. Neither in men nor in women iodine excretion was associated with TSH or fT4 levels.
Conclusion
Our data demonstrate that iodine supply in the Western Part of Germany has normalized. In men, elevated TPO antibodies were associated with high iodine excretion.

P739

Interleukin-6 is not essential for bone turnover in hypothyroid mice

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Interleukin-6 (IL-6) has been shown to be involved in the pathogenesis of several bone diseases characterized by an imbalance between bone resorption and formation. Lately we have published our data suggesting an important role of IL-6 in thyrotoxicosis-related osteoporosis in mice (*Horm Metab Res* 2007 **39** 1–5).
Aim
The aim of the study was to estimate serum markers of bone turnover: osteoclast-derived tartrate-resistant acid phosphatase form 5a (TRACP 5b) and osteocalcin in IL-6-deficient mice to assess the role of IL-6 in bone metabolism in hypothyroidism in mice.
Material and methods
C57BL/6J (wild-type; WT) and C57BL/6J$^{IL6-/-Kopf}$ (IL-6 knock-out; IL6KO) mice randomly divided into 4 groups with 10 in each one: 1/WT mice in hypothyroidism (WT-ht), 2/WT controls, 3/IL6KO mice with hypothyroidism (IL6KO-ht) and 4/IL6KO controls. Experimental model of hypothyroidism was induced by intraperitoneal injection of propylthiouracyl. The serum levels of TRACP 5b and osteocalcin were determined by ELISA.
Results
Serum concentrations of TRACP 5b (median and interquartile ranges) were significantly decreased in both groups of mice with hypothyroidism: WT (3.2 (2.5–4.7) U/l) and IL6KO (2.6 (1.8–3.5) U/l) as compared to the respective controls. Similarly, serum osteocalcin levels were significantly reduced in both groups of mice in experimental hypothyroidism: WT (25.8 (23.0–28.2) ng/ml) and IL6KO (21.5(19.0–24.6) ng/ml) in comparison to the respective controls. There were no significant differences in bone turnover markers between IL6KO and WT mice both in hypothyroid and control animals.
Conclusions
The results of the present study suggest that IL-6 does not play an important role in bone turnover in both euthyroid and hypothyroid mice.

P740

Serum ghrelin levels are increased in hypothyroid patient and become normalized after L-thyroxin treatment

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Context
An interaction between gut-derived ghrelin, which is implicated in the regulation of short- and long-term energy balance, and thyroid function has previously been reported in patients with hyperthyroidism, in whom ghrelin levels are reversibly suppressed. In the present study, we measured total serum ghrelin levels and pertinent metabolic indices in hypothyroid patients before end after L-thyroxin replacement.
Patients and methods
Eleven patients were examined twice: 1) in the hypothyroid state, and 2) after at least 2 months of euthyroidism. Ten healthy subjects served as a control group. Ghrelin was measured in conjunction with indirect calorimetry and a hyperinsulinemic euglycemic clamp.
Results
Serum ghrelin levels were increased by 32% under basal conditions in the hypothyroid state (PRE) as compared to post treatment (POST) (pg/ml): 976.4 ± 80.8 vs 736.8 ± 67.1 ($P < 0.001$). This difference prevailed during the clamp but a decline was observed in both states: 641.4 ± 82.2 vs 444.3 ± 66.8 μg/ml ($P = 0.005$). The hypothyroid state was associated with decreased resting energy expenditure, increased respiratory quotient and insulin resistance. Serum ghrelin levels as well as the metabolic aberrations became normalised after L-thyroxin replacement as compared to the control subjects.
Conclusion
Serum ghrelin levels are reversibly increased in hypothyroid patients. It remains to be investigated whether this represents a direct effect of iodothyronines or a compensatory response to the abnormal energy metabolism in hypothyroid patients.

P741

Pharmacokinetics of digoxin in hyperthyroidism: effect of methimazole and acebutolol

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Hyperthyroid patients show an impaired response or even resistance to digoxin treatment.
Objectives
1. Are there any differences in the pharmacokinetics of a single oral dose of digoxin between hyperthyroid and euthyroid patients? 2. Does simultaneous administration of digoxin and methimazole or digoxin and acebutolol affect the pharmacokinetics of a single oral dose of dogoxin? 3. Does methimazole-induced euthyroidism change the pharmacokinetics of a single oral dose of digoxin? 4. Does acebutolol, which ameliorates symptoms of hyperkinetic circulation change the pharmacokinetics of a single oral dose of dogoxin?
Design and method
The subject of the study were 28 patients with hyperthyroidism and 15 healthy persons.We evaluated the pharmacokinetics of a single oral dose of digoxin. Twelve methimazole treated patients were re-assessed once they had become euthyroid. Sixteen acebutolol-treated patients were re-asesed once symptoms of hyperkinetic circulation had subsided.Moreover we evaluated pharmacokinetics of a single dose of digoxin after simultaneous administration of digoxin and methimazole or digoxin and acebutolol.
Results
Hyperthyroid patients showed significantly lower serum digoxin concentrations, shorter $T^{1}/_{2}$ beta and a significantly smaller area under the concentration curve that the control group. Administration of methimazole did not affect digoxin pharmaco-kinetics, administration of acebutolol resulted in an increased serum digoxin concentration and in a longer time to the peak serum level of digoxin in comparison with the control group.
Conclusions
In hyperthyroid patients the pharmacokinetics of a single oral dose of digoxin does differ from that observed in healthy subjects. Acebutolol but not methimazole do alter digoxin pharmacokinetics in hyperthyroid patients.

P742

Iodine metabolism and effect of ^{131}I therapy in Graves' disease

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The factors influencing the final outcomes of ^{131}I therapy are indefinitely known, besides its practical use since more than 60 years. One of the considered factors affecting the results of ^{131}I therapy is iodine metabolism.
Aim
The aim of the study was the evaluation of the iodine uptake and the effective half-life of ^{131}I influence on the result of radioactive iodine therapy in patients treated due to Graves' hyperthyroidism.
Material and methods
A hundred subjects (84 females and 16 males) aged 27–76 with diagnosed Graves' disease, were enrolled. In all patients, the thyroid technetium-99m scan and determination of the serum levels of FT3, FT4, TSH, TSHRAb were performed. Iodine uptake was measured at 24 h, 48 h, then the half-life has been determined. The therapeutic activity of ^{131}I was calculated according to Marinelli formula. After the 12th months follow up period, the thyroid function has been estimated.
Results
The levels of thyroid hormones and TSH before therapy were: FT3 5.7–74.8 pmol/l, FT4 10.3–90 pmol/l, TSH 0.02–0.08 mIU/l.
After 24 h, radioiodine uptake ranged between 18 and 89%, but after 48 h between 17 and 83%. The effective half-life range was from 1.2 to 8.1days.
The administered therapeutic activities ranged between 148 and 1113.7 MBq.
After 1 year follow-up, the effective ^{131}I therapy was confirmed in 60 patients (29 euthyreosis, 31 hypothyreosis), but in 40 patients hyperthyreosis still remained.
Statistical analysis revealed no correlations between 24-h and 48-h radioiodine uptake as well as T1/2 and the result of ^{131}I therapy.
Conclusions
The iodine uptake as well as the effective half-life of ^{131}I did not influence the result of ^{131}I therapy.

P743

Analysis of concomitant thyroid pathology in patients with thyroid hemiagenesis

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Introduction
Thyroid hemiagenesis (TH) is a rare inborn anomaly of unclear importance. The aim of the study is to describe clinical, hormonal and autoimmunological profile of patients with TH.
Material and methods
The studied group consisted of 20 subjects (4 men), diagnosed of having TH at the age from 15 to 63. Laboratory tests, including measurement of serum concentration of thyroid-stimulating hormone (TSH), free thyroxine (FT_4), free triiodothyronine (FT_3), thyrotropin receptor antibody (TRAb), antithyroid peroxidase antibody (aTPO), antithyroglobulin antibody (aTg) as well as thyroid scintigraphy and ultrasound examination (US), were performed. The data were analyzed with regard to the circumstances and the age of establishing diagnosis, gender, side of agenesis, associated thyroid developmental anomalies, US abnormalities, thyroid volume, hormonal function, concomitant thyroid diseases, administered therapy and the follow-up period.
Results
The left to right sided agenesis ratio was 4:1, with associated isthmus agenesis in 7 (35%). Compensatory enlargement of thyroid lobe was found in 14 cases (70%) and was not correlated to TSH concentration. Thyroid volume was significantly lower in the young (<25 years); $P=0.0035$. US performed in 17 patients, revealed abnormal echogenity in 13 (76.5%) and focal lesions (nodules and/or cysts) in 8 (47.1%), all benign by fine-needle aspiration biopsy and significantly less often found in younger subjects (<25 years); $P=0.044$. At diagnosis, 8 patients (40%) were biochemically euthyroid (TSH $=2.0\pm0.38$ μIU/ml), 7 (35%)-hypothyroid and 5 (25%)-hyperthyroid. Thyroid autoantibodies were detected in 10 subjects (50%), more frequently among patients >40 years ($P=0.069$).
Conclusions
The study indicates high prevalence of US abnormalities and thyroid autoimmunological disorders of frequency increasing with age, in subjects presenting TH, probably as a consequence of prolonged TSH overstimulation. Therefore, systematic observation and hormonal treatment, if required, is recommended in all detected cases.

P744

Assessment of VEGF, VEGF receptor, EGF and endostatin concentrations in patients with endemic goiter

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Tumor angiogenesis is believed to result from an imbalance of pro- and anti-angiogenic factors. Angiogenesis is an important component in the development of thyroid goitre. Vascular endothelial growth factor (VEGF) and epidermal growth factor (EGF) represents a family of specific endothelial cell mitogens involved in normal angiogenesis and in tumor development. Consequently, a panel of anti-angiogenic factors was addressed in a representative sample of VEGF receptor-2 (VEGFR-2) and endostatin. Mechanism through which endostatin and VEGFR-2 suppressed angiogenesis was induction of endothelial cell apoptosis and inhibition of endothelial migration. The aim of our study was to evaluate the concentrations of VEGF, its soluble receptor, EGF and endostatin in peripheral blood of patients with endemic goitre. The study comprised 91 patients with nodular goiter, 34 with parenchymatous goiter and 51 persons without pathology of thyroid (control group). VEGF, VEGFR-2, EGF and endostatin were determined by Human Immunoassay – Quantikine® ELISA (R&D Systems, Minneapolis, USA). The highest concentrations of VEGF and EGF was demonstrated in nodular goitre in the medium level in parenchymatous goitre and the lowest level in subjects with normal thyroid. Respectively, VEGFR-2 and endostatin were lower in patients with goiter than in the control group, but without significant differences. In the group with goiter, significant Spearman correlation between concentration of endostatin and iodine urine excretion was observed ($P<0.02$). In conclusion, the observed misbalance between inhibitors and accelerators of angiogenesis could be an element of goitrogenesis.

P745

Surgery, antithyroid drugs, or glucocorticosteriod in amioadarone-induced hyperthyroidism (AIH) in patients with low iodine uptake: that is the question

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Amiodarone is frequently used in cardiac patients but has side effects which effects the thyroid due to its high iodine content. The use of radioiodine in AIH with low iodine uptake is controversial. In these patients therapeutic choices for refractory cases include either: surgery, antithyroid drugs, or glucocorticosteriods.
What are the treatment choices in elderly patients which can't be operated on, agranulocytosis is present, or have toxic liver failure after methimazol therapy?
Aim
The aim of study was the evaluation of radioiodine therapy results in AIH patients with low radioactive iodine uptake (RAIU).
Patients and methods
We examined 9 patients with AIH, 1 female (11.1%), and 8 males (88.9%) aged from 63 to 78 years. ($x\pm$S.D.: 69.2 ± 5.5 years). The therapy including amiodarone was essential for the underlying heart disorder. Radiotherapy was the necessary medical choice because of: in two cases – agranuloccytosis post antythyroid drugs, additionally in two cases with diabetes mellitus (against glucocorticoid therapy), and five hepatic failure. In all of them, surgical intervention was excluded. The diagnostic procedure included baseline thyroid function test (fT3, fT4 and TSH levels), thyroid autoantibodies (TgAb, TPOAb, TRAb), thyroid ultrasound, thyroid scan, and RAIU. RAIU in all cases was below 10% at 24 h.
Results
Serum values of TSH, TgAb, TPOAb, TRAb were undetectable. Serum fT4, fT3 were as follows: fT4 12.4 to 49 pmol/l (mean: 28.4 ± 13.6); fT3 3.9 to 6.8 pg/ml (mean: 5.7 ± 1.4). Mean thyroidal 5-h and 24-h RAIU values were 5.1 ± 1.0 and 8.2 ± 1.4%, respectively. In every patient, an ablative dose of 131I (22 mCi = 814 MBq) was administered.
Conclusion
Iodine therapy can be a useful therapeutic method in every case with low RAIU and in which other treatments (surgery, antytyroid drugs, glucocorticoid) are contraindicated if the dose used is ablative.

P746

Incidental thyroid carcinomas: do they have a different clinical aggressiveness?

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With the increasing use of thyroid ultrasound (US), the recognition of thyroid nodules in a large proportion of apparently healthy subjects has become common. Moreover, some autopsy series have shown that papillary cancer measuring 10 mm or less, the so-called papillary thyroid microcarcinoma (PTMC), is a very frequent incidental finding. Because PTMC is being increasingly discovered, it is important to ascertain whether PTMC may exhibit heterogenous clinical and pathological features, associated with different aggressiveness. To test this hypothesis, we examined 122 consecutive cases of thyroid cancer (31 incidental and 91 non incidental) to find potential clinical and pathological findings that could be predictive of their aggressiveness behaviour. The cancers are considered invasive in the presence of the following conditions: capsular infiltration, stage N or M more or equal to 1. To identify clinical and histological factors related to more invasive cancer, a logistic regression model was performed. All independent variables were considered in the multivariate logistic regression with stepwise selection; variables entered in the model at significance level 0.05.
Results
In the group of 31 patients with true incidental cancer, 20 of them (64.5%) had a diameter <10 mm (PTMC) with a statistically significant difference ($P<0.0001$) as compared to the group of non incidental thyroid carcinomas. When size and incidental discovery were examined in relation to the invasiveness, after adjusting for multiple comparisons, a statistically significant difference ($P=0.027$) was found between PTMC incidentally discovered (4/20), which were less invasive, and the group with non incidental discovery and diameter >10 mm (39/71), which were more invasive. The logistic regression analysis showed both in

univariate and in multivariate analysis that the papillary histotype and the diameter $\leq$10 mm (incidental or non incidental) resulted less aggressive.
Conclusions
We have demonstrated that the papillary histotype and the diameter $\leq$1 cm resulted as the only protective factors in term of invasive behaviour. It is likely that in the near future molecular studies will be able to discriminate aggressive PTMC from those with an indolent clinical course.

P747

Thyroid disorders in pregnancy

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After diabetes, thyroid disorder is the second most common endocrine disorder in women of reproductive age. Thyroid disorders in pregnancy have been proven to have adverse maternal and foetal outcome if not treated adequately and timely.
Aim
The aim of this audit was to assess management and therapeutic interventions and outcomes of pregnancies complicated by thyroid disorders.
Method
A retrospective case notes analysis of 35 pregnancies with thyroid disorders booked in antenatal clinic at Good Hope Hospital, Sutton Coldfield in 1996 was performed. Parameters included were prepregnancy counselling, thyroid function tests assessment, change in treatment instituted, outcome of pregnancy. Standard laboratory non-pregnant reference ranges were used for thyroid function test interpretation in all three trimesters.
Result
Of 35 pregnancy 32 (91%) had hypothyroidism and 3 (9%) had hyperthyroidism. Prepregnancy counselling was provided to 13% of pregnant women with thyroid disorders. In hypothyroid group medication increased in 21% and decreased in 3%. In hyperthyroid group, Propylthiouracil started in 2 (66%) and decreased and stopped in 2 (66%).
Conclusion
With appropriate and timely adjustment in therapy majority of pregnancy had good outcome. Usually in pregnancy with hypothyroidism, the dose of levothyroxine needs increasing but in one of our pregnant woman it was decreased. We need consensus on reference range for interpretation of thyroid function test in different trimesters of pregnancy.

P748

TSH screening and thyroid disease comorbidities

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Background
Thyroid diseases are commonly encountered in the population though it is unanswered for whom screening tests are useful. We aimed to see the value of TSH screening and to find out comorbidities of any thyroid diseases among these patients who were diagnosed by screening.
Method
All consecutive patients (*n*: 796) who admitted to outpatient Clinics of Internal Medicine, between 1 and 31 January 2007 were recruited in the study. Age, gender, final diagnosis were recorded. TSH levels were measured by chemiluminesance method (Roche Diagnostics GmbH, Mannheim, Germany). When they were found out of reference values, after two weeks, TSH levels were repeated and FT_3 and FT_4 levels were also measured by the same method.
Results
Data were collected from 796 patients (393 male, 403 female). Thirty-seven patients had been followed up with known thyroid disease. Among these 10 were hyperthyroid, 10 were hypothyroid despite treatment and 17 were euthyroid. 55 (7.2%) subclinical hyperthyroidism (TSH: 0.12 ± 0.08 μIU/ml), 48 (6.3%) subclinical hypothyroidism (TSH: 6.24 ± 2.76 μIU/ml) and 4 (0.5%) overt thyroid disease (3 hypothroidism and 1 hyperthyroidism) were diagnosed. Subclinical hyperthyroidism and hypothyroidism rates were found similar with overt thyroid disfunction. Subclinical hypothyroidism was more common among women than men (68 vs 32%, $P=0.001$) although there was not such a relation for subclinical hyperthyroidism ($P=0.919$). With regard to comorbidities we could not find a significant relation between cardiovascular disease or hypertension in both subclinical thyroid diseases ($P>0.05$). There was a slight increase of diabetes mellitus in subclinical hyperthyroid group ($P=0.08$), compared to subclinical hypothyroid group ($P=0.367$).
Conclusion
Although it is not clearly supported by our study, considering the burden of comorbidity of thyroid dysfunction, we recommend TSH screening for patients with diabetes and cardiovascular disease, especially for women.

P749

ProEGF cytoplasmic domain (proEGFcyt)-mediated up-regulation of SNAP25 decreases cathepsin-L secretion and elastinolytic activity in human thyroid carcinoma cells.

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The cytoplasmic domains of EGF-like ligands have important biological functions. Stable transfectants of the human follicular thyroid carcinoma cell line FTC-133 over-expressing the cytoplasmic domain of proEGF (FTC-133-proEGFcyt) demonstrated a transcriptional up-regulation of the lysosomal hydrolases cathepsin- (cath-) B and -D and alterations in the processing of cath-L protein. Cath-L has strong elastinolytic activity and was the only of the three cathepsins to be secreted by FTC-133 transfectants. FTC-133-proEGFcyt clones displayed a markedly reduced ability to migrate through elastin matrices when compared with mock transfectants (empty plasmid) or a natural proEGFsplice mutant construct. Decreased migration through elastin matrix coincided with a reduction of cath-L secretion in FTC-133-proEGFcyt clones. When incubated with a specific cath-L inhibitor, FTC-133-proEGFsplice and mock transfectants showed a similar reduction in elastinolytic activity implicating cath-L to be largely responsible for the elastinolytic activity in our elastin micration assays. Down-regulation of cath-L in FTC-133-proEGFcyt coincided with an upregulation of the t-SNARE component SNAP25 as determined by specific siRNA knock-down of SNAP25. Incubation of FTC-133-proEGFcyt with soluble EGF reduced SNAP25 protein levels in proEGFcyt transfectants and, thus, reversed the inhibitory actions of proEGFcyt on elastinolytic migration. This antagonistic EGF action was mediated by the EGFR. In summary, we have identified proEGFcyt as a novel regulator of the function of the t-SNARE membrane-vesicle fusion complex involved in the exocytosis/secretion of elastinolytic cath-L. This mechanism facilitates the suppressive role of proEGFcyt domain on thyroid carcinoma cell elastinolytic activity and invasiveness. Partly supported by Krebshilfe.

P750

Impact of Cinacalcet treatment on health related quality of life (HRQOL) in patients with primary hyperparathyroidism (PHPT) who have failed or in whom parathyroidectomy (PTX) is contraindicated

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Hypercalcemic complications of PHPT include renal, cardiovascular, gastrointestinal, neuromuscular and neuropsychiatric issues, all potentially impacting negatively on HRQOL. As control of the hypercalcemia associated with PHPT might improve HRQOL, the SF-36 and a 6-item scale on cognitive functioning (CF) were included in a phase 2, single-arm study evaluating the ability of cinacalcet to control serum calcium levels in 17 patients with intractable PHPT. Mean $\pm$ S.E.M. calcium in these patients fell significantly from 3.2 ± 0.05 to 2.6 ± 0.08 mmol/l after up to a 16-week titration phase. The SF-36 scales are standardized so that the general population mean is 50. The CF scale is scored from 0 to 100. Higher numbers indicate better HRQOL. Mean change from baseline to end of the treatment phase for these patients was calculated for each scale. Numerically scores for all scales improved during treatment. For the SF-36, the gain ranged from 3.0 to 7.7 points. For the CF, it was 11.8 (Table).

HRQOL scales	Mean (S.E.M.) score at baseline	Mean change from baseline (95% confidence interval)
Physical functioning (PF)	34.3 (3.2)	4.6 (2.0, 7.2)
Body pain (BP)	42.8 (3.1)	5.1 (0.7, 9.6)
Role limitations-physical (RP)	35.0 (2.6)	3.3 (−1.1, 7.7)
General health (GH)	40.9 (2.5)	3.0 (−2.3, 8.3)
Social functioning (SF)	36.7 (3.6)	6.9 (−2.1, 15.9)
Vitality (VT)	37.8 (2.6)	5.6 (−2.7, 13.8)
Role limitations-emotional (RE)	37.4 (3.4)	7.7 (0.6, 14.9)
Emotional well being (EW)	38.5 (3.3)	7.5 (−0.1, 15.1)
Cognitive functioning (CF)	61.2 (5.7)	11.8 (−2.4, 25.9)

Treatment with cinacalcet may lead to improvement in HRQOL and functioning in patients with PHPT who have failed parathyroidectomy or in whom surgery is contraindicated. Larger, randomized studies are needed to confirm these findings.

P751

Same aspects regarding immunohistochemical diagnosis of papillary thyroid carcinoma

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The study evaluates the expression of same immunohistochemical (IHC) markers (Ki-67, PCNA, CK-19, HBME-1 and Galectin 3, in the diagnosis of differentiated thyroid carcinomas.
Material and methods
The study group comprised 57 patients with thyroid tumors; aged 17–71 years (mean age 48.96 years). All cases were evaluated before surgical treatment by means of clinical examination, thyroid ultrasonography and fine needle aspiration biopsy (FNAB).
IHC staining was performed (on formalin fixed paraffin-embedded tissue) in 37 cases of papillary thyroid carcinoma – PTC (classical form, variants and microcarcinomas), 6 cases of follicular adenoma – FA, 4 cases of follicular thyroid carcinomas – FTC, 5 cases of neoplasia with uncertain malignant potential and 5 cases of Hurthle cell tumors (carcinomas and adenomas).
The obtained results were expressed semi quantitatively.
Results
All studied markers were expressed in the tumor cytoplasm, with no or weak expression in benign tumors and diffuse and strong in malignant ones.
Twenty-two cases with carcinomas were positive for Ki67 and 27 for PCNA.
CK19 was detected in all PTCs, 2/4 FTCs and 2/6 FAs.
Galectin 3 expressed in 32/37 PTCs, all FTCs and 4/5 neoplasms with uncertain malignant potential. HBME-1 showed a highly specificy for PTC.
Conclusions
HBME-1 and CK19 proved to be helpful markers in diagnosis of PTC.HBME-1 helps the differential diagnosis between follicular variant of PTC (FVPTC) and FTC.
Galectin 3 was helpful in the differential diagnosis between FTC and FA and in the diagnosis of tumors with uncertain malignant potential.
The combined use of HBMB1, Galectin 3 and CK19 seems to increase the specificity and diagnostic accuracy of the IHC method.

P752

Low-T3 syndrome in chronic obstructive pulmonary disease and heart surgery patients; evaluation of plasma antioxidant systems

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Previously, we demonstrated an inverse correlation between CoenzymeQ10 (CoQ10) and thyroid hormones, suggesting its usefulness as index of thyroid hormone tissue effect. A low-T3 syndrome, observed in chronic diseases, is considered an adaptive mechanism and its treatment is still debated. To evaluate the metabolic status of these patients and antioxidant vs energetic role of CoQ10, we studied 32 patients, with chronic obstructive pulmonary disease (COPD), comparing respiratory indexes, thyroid hormones, CoQ10 (also corrected for cholesterol levels) and Total Antioxidant Capacity (TAC) in patients with low ($n=12$) or normal ($n=20$) fT3 concentrations. Another low-T3 model was represented by five patients studied after major heart surgery (HS). Twenty-one normal subjects were studied as controls. CoQ10 were assayed by HPLC; TAC was determined using the system metmyoglobin-H_2O_2, which interacting with the chromogen ABTS generates a radical spectroscopically revealed; the latency time (Lag) in the appearance of radical species is proportional to the antioxidant content. CoQ10/Cholesterol ratio values were significantly higher in COPD with low- vs normal-fT3 (CoQ10: 0.88 ± 0.06 vs 0.83 ± 0.07 μg/ml; CoQ10/Cholesterol 239.7 ± 28.5 vs 185.1 ± 14.0 nmol/mmol, $P<0.05$); TAC showed opposite pattern (54 ± 6 vs 64 ± 2 s). Similarly in post-surgical cardiac patients, all exhibiting low values of fT3, CoQ10 levels were in the hypothyroid range (CoQ10: 0.97 ± 0.01 μg/ml; CoQ10/Cholesterol: 216.6 ± 3.85 nmol/mmol) despite the fact cardiac diseases are well known to be associated with low CoQ10.
These data suggest that low fT3 levels are accompanied by indexes of true hypothyroidism in COPD and HS, supporting the need for replacement therapy. The different TAC pattern suggests that elevated CoQ10, despite oxidative stress, expresses a reduced tissue utilization.

P753

Thyroid function and glucose tolerance in pregnancy: THYROMOBIL pilot study in Poland

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Aim of the study was
1. To assess the thyroid function in pregnancy in the course of effective iodine prophylaxis model introduced in 1997, based on obligatory salt iodization.
2. To exam glucose tolerance and insulinemia in pregnancy.
Methods
TSH, fT4, aTPO, urine iodine concentration, usg thyroid volume and homogeneity, glucose and insulin during OGTT, blood pressure, BMI, and thyroid function and glucose metabolism family history questionnaire, use of iodine supplements questionnaire were examined in healthy pregnant women ($n_{pilot}=103$, recruited from the patients of Department of Gynecology and Obstetrics, pregnancy trimester I-37%, II-47%, III-16%) in THYROMIBIL action.
Results
Goiter prevalence was 3.1%. Solitary thyroid nodules and abnormal homogeneity were detected in 17.5 and 20.5% of pregnant women respectively. TSH over 2.5 μj/ml, decreased fT4 and increased aTPO were detected in 18.7, 14.1 and 9.6% of probants respectively. Sixty percent of pregnant women supplemented diet with 150–200 μg of iodine, only 26% had no supplementation. Family history of thyroid dysfunction was observed in 28.9%. Increased fasting glucose was found in 5%, and impaired glucose tolerance was observed in 3.3% of pregnant women. Mean fasting and after glucose load insulin was 12.1 μj/ml (S.D.=12) and 37 μj/ml (S.D.=22) respectively. Increased systolic and diastolic blood pressure was found in 5.2 and 12.4% of probants.
Conclusions
In spite of obligatory model of iodine prophylaxis still high incidence of nodules in thyroid was observed. More than 70% of women had additional iodine supplementation.
Abnormal glucose metabolism and hypertension in pregnancy requiring medical consultation was observed in more than 5% of women.
Our study confirms usefulness of THYROMOBIL model in assessment of thyroid function and glucose metabolism in pregnancy.

P754

Interference in assay of thyroid hormones due to auto-antibodies against thyroxine and triiodothyronine: report on two patients with Hashimoto's thyroiditis

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	TSH (EQLA)	FT4 (EQLA)	FT4 (CMIA)	AAcT4 (%^{125}I-T4)	FT3 (EQLA)	FT3 (CMIA)	AAcT3 (%^{125}I-T3)	Sub-α (IRMA)	SHBG (QLA)
1	25	2.3	0.9	57.3	3.6	3.22	31.8	0.5	75
2	98.4	3.08	0.55	67.5	3.16	1.53	10.4	0.68	59
nv	0.38–4.84 μU/ml	0.8–2 ng/dl	0.7–1.48 ng/dl	<1.6%	1.8–4.6 pg/ml	1.71–3.71 pg/ml	<2%	0–0.9 mU/ml	11–124 nmol/l

Introduction
Characteristically the discordant thyroid function test has been attributed to TSH-producing hypophyseal adenoma, familial dysalbuminemic hyperthyroxinemia and thyroid hormone resistance syndrome. We presented 2 cases with raised peripheral thyroid hormones with detectable TSH due to the presence of auto-antibodies against the peripheral thyroid hormones (PTAAb).
Subjects and methods
A 24-year-old woman (1) and a 79-year-old woman (2) with auto-immune primary hypothyroidism diagnosed 4 and 3 years ago, with symptoms and signs of clinical hypothyroidism and without levothyroxine treatment was referred to our centre after a finding of discordant thyroid function values (Table). Hormonal and radioimmunoprecipitation studies. Subunit-α was determined by IRMA (Immunotech de Beckman Coulter©), SHBG by QLA (Immulite 2000 (DPC©), TSH was determined by EQLA, following series dilution (1/2, 1/5) of the sample. Samples were sent to another laboratory for determination of TP by 'two-step' immunoassay (CMIA, Architect (Abbott Lab©). TPAAb was determined by incubating samples with I^{125}-T4 and I^{125}-T3, followed by precipitation in polyethylene–glycol. Precipitate count was obtained using a gamma-counter (Packard Cobra). Euthyroid patients with no auto-immune pathology were used as controls.
Results
The presence of PTAAb was detected in both cases (table), confirming the initial diagnosis of primary hypothyroidism.
Conclusion
The presence of PTAAb is an unusual cause of discordant thyroid function values that it must have in mind to the correct management of thyroid disease.

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Image-guided radionuclide therapy of malignant melanoma following tumor-specific sodium iodide symporter (NIS) gene transfer

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In its early stages, malignant melanoma can be cured by surgical resection, but once it has progressed to the metastatic stage it does not respond to current therapies. Therefore, the development of novel treatment strategies, such as gene therapy, is urgently needed. To investigate an alternative approach, we examined the feasibility of NIS-mediated radionuclide therapy (^{131}I, ^{188}Re) of malignant melanoma following human sodium iodide symporter (NIS) gene transfer using a melanoma-specific tyrosinase-promoter construct to target NIS expression to melanoma cells. For this purpose, a human melanoma cell line (Lu1205) was stably transfected with hNIS cDNA under the control of the tyrosinase-promoter. The stably transfected Lu1205 cell line showed perchlorate-sensitive iodide uptake activity which was sufficiently high for a therapeutic effect of ^{131}I as shown in an *in vitro* clonogenic assay. After injection of 18.5 MBq, ^{123}I NIS-transfected Lu1205 xenografts in nude mice accumulated approximately 30% ID/g ^{123}I with a biological half-life of approximately 11.1 h and an effective half-life of 10.5 h. In comparison, tumoral accumulation of ^{188}Re, which is also transported by NIS, was 15%ID/g with a biological half-life of approximately 15 h and an effective half-life of 8.2 h. After administration of a therapeutic dose of 55.5 MBq, ^{131}I or ^{188}Re NIS-expressing tumors showed an average tumor volume reduction of approx. 50 and 20%, respectively, while control tumors continued their rapid growth exponentially.
In conclusion, a significant therapeutic effect of ^{131}I and ^{188}Re has been demonstrated in melanoma cells following tyrosinase-promoter-directed NIS gene transfer *in vitro* and *in vivo* with higher efficacy of ^{131}I. This study demonstrates the potential of NIS-mediated radionuclide therapy of melanoma following tumor-specific NIS gene transfer offering an innovative strategy for melanoma therapy.

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The role of elevated microvessel density in the metastatic potential of papillary thyroid carcinoma

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Introduction
The value of microvessel density (MVD) as a prognostic factor in thyroid carcinomas, and its role in the development of metastasis, remain controversial.
Aim
Determination of the relationship between MVD, the histological subtype of PTC, the presence or absence of metastasis in cervical lymph nodes (LN), the recurrence of the disease and AMES prognostic index.
Material and method
The study included 32 patients with PTC (mean age of 48.8 years) divided into 2 risk groups, depending on AMES prognostic index.
Tissue sections fixed in formalin 10% and paraffin-embedded were colored with monoclonal antibody anti-CD31, clone JC/70A, using the LSAB technique and visualization with DAB.
Results
We noted: 1) Classic PTC in 20 cases (62.5%), follicular variant of PTC (FVPTC) in 6 cases (18.75%), PTC with diffuse sclerosis (PTCDS) in 2 cases (6.25%) and PTC with tall cells in 4 cases (12.5%); 2) 17% micropapillary carcinomas ($\leq$1 cm ϕ), 75% with ϕ between 1 and 4 cm and 8% with $\phi >$4 cm; 3) extrathyroid extension in 8 cases (25%), vascular invasion in 8 cases (25%), perineural invasion in 4 cases (12.5%), lymphatic invasion in 42% and multicentric dissemination in 45%; 4) mean MVD of 84 (64–114) in PTC without LN metastasis and 104 in PTC with positive LN; 5) a higher mean MVD in FVPTC (independent of metastasis) compared with classic PTC (98 vs 84), PTC with tall cells (80) and PTCDS (68); 6) mean MVD of 84 in patients $\leq$45 years and of 93 in those $>$45 years; 7) slightly higher mean MVD in the group of patients with recurrences.
Conclusions
Elevated mean MVD in PTC with LN metastasis and in patients with recurrent disease indicates the metastatic potential of these tumors; we did not note a relationship between MVD and the AMES prognostic index.

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Thyroglobulin measurement in fine-needle aspirates of cervical lymph nodes in diagnosis of metastic differentiated thyroid cancer

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Introduction
Serum thyroglobulin (Tg) measurement is the marker of differentiated thyroid carcinoma (DTC) after total thyroidectomy, but its value is limited by the interference of anti-Tg antibodies (TgAb). Detection of Tg in fine-needle aspiration biopsy (TG-FNAB) washout fluid is used recently to identify neck DTC metastases, but the interference of TGAb-FNAB in the procedure is unknown.

Objectives
To evaluate the utility of TG-FNAB for detecting cervical lymph node metastases from DTC. To evaluate the presence of anti-Tg antibodies in this fluid.
Patients and methods
An ultrasound-FNAB was done in all patients with suspected recurrence or metastasis of CDT treated at the Alicante General University Hospital since June 2006 since November 2007. Tg and TgAb serum and liquid-FNAB was assayed by chemiluminescent assays (IMMULITE 2000, Siemens©). The aspirated material was cytologicaly examined and were reported by an experienced cytologist.
Results: Table.
Conclusions
Tg-FANB could be a marker in the early diagnosis of DTC recurrent or metastatic. The clinical performance of Tg-FANB fluid appears to be not affected by TgAb-FANB, on the observed data.

	FANB washout fluid		Serum		
	Tg (ng/ml)	TgAb (UI/ml)	Tg (ng/ml)	TgAb (UI/ml)	Cytology
Group 1 $n=4$	3392	<5	<0.2	<5	Metastatic DTC
	21 460	<5	1.1	<5	Metastatic DTC
	1271	<5	0.3	<5	Metastatic DTC
	470	<5	0.8	<5	Metastatic DTC
Group 2 $n=4$	<0.2	<5	<0.2	6.2	No malignancy
	<0.2	<5	<0.2	<5	No malignancy
	<0.2	<5	<0.2	6.8	No malignancy
	<0.2	<5	<0.2	9.2	No malignancy
Group 3 $n=2$	<0.2	<5	0.3	13.1	Inadequate
	<0.2	<5	<0.2	5.3	Inadequate
Group 4 $n=2$	0.7	<5	18.1	9.3	No malignancy
	64	<5	16	20.3	No malignancy

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Visual evoked potentials in diagnosis of orbitopathy during the course of Graves-disease

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Graves disease (GD) is autoimmunological disorder leading most often to hyperthyroidism and invasive-edema opthalmopathy (Graves opthalmopathy – GO).
Aim
Estimation of the relation of visual evoked potentials (VEP) results with the indicators of activity and advancement of the progress of GO.
Materials and methods
The examined group consisted of 45 patients between the age of 24 and 77, hospitalized in the Department of Endocrinology and Metabolism. Duration of GD from the first clinical signs to the start of treatment took between 3 months to 20 years. Changes of the eye due to GO occurred from 3 months to 6 years. VEPs were carried out according to recommendations of the International Federation of Clinical Neurophysiology. Latencies and amplitudes of VEP components were compared to normal values.
Results
According to the NOSPECS scale 2 people showed no visual symptoms. In 5 cases, class 1 or 2 was diagnosed. Furthermore, in 38 patients classes between 3 and 6 were observed. Possible loss of vision due to visual nerve damage (class 6) was found in only 4 patients. The CAS criteria in 8 patients was equal to 0, and the remaining 14 patients from 1 to 3. Active GO was diagnosed in 23 patients. In 35 (77.8%) patients, abnormal VEPs were recorded. Normal parameters of VEP were observed in only 10 (22.2%) patients. These were patients with inactive or mild processes involving eye balls. Changes in latency of P100 increased from 123 ms in mild, to 127 ms in intermediate, to 129 ms in intense GO. Referring to the control group a statistical change was observed in latency of P100 and N145. They were extended already in mild occurrence of GO which confirmed subclinical visual nerve involvement.
Conclusions
VEP can be helpful in diagnosing visual nerve neuropathy in patients with GD. The clinical interpretation of changes of P100 latency is very important in patients with GO.

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Some data about the epidemiology of thyroid cancer in Albania

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Introduction
Aim: the aim of our study was to give some data about the epidemiology of thyroid cancer in our country.
Material and methods
We studied patients diagnosed with thyroid cancer during January 1998–October 2007.
Results
We studied 179 patient from 13 to 80 years diagnosed with thyroid cancer during January 1998–October 2007. Median age at dignoses was: 46.2 ± 14.7 years. According to the gender: females were 139 (77.5%); males 40 (22.5%); ratio F/M 3.4: 1. Distribution according to the type were: papillary 95 (52.9%) folliculary 58 (32.7%) medullary 8 (4.4%) anaplastic 13 (7.2%) lymphoma 3 (1.7%) other type 2 (1.1%). Pre surgical diagnosis were: multinodular goitre 93 (51.9%); cold nodule 69 (38.8%); hot nodule 6 (3.5%); Graves disease 2 (1.1%); thyroiditis chronic Hashimoto 1 (0.5%); suspect thyroid cancer 7 (4%); lymphoma 1 (0.5%). Distribution according to the years of the study: cytological evaluation were done in 53 cases (29.60%) and the results were: negative 9 (16.9%); suspicious 13 (24.6); malignant 31 (58.5%). Distribution of cases according to the years were: 1998 6 cases; 1999 13 cases; 2000 14 cases; 2001 14 cases; 2002 17 cases; 2003 16 cases; 2004 12 cases; 2005 25cases; 2006 36 cases; 2007 25 cases. Distribution according to the age group: 10–20 years old 7 cases 20–30 years old 18 cases; 31–40 years old 42 cases; 41–50 years old 37 cases; 51–60 years old 42 cases; >60 years old 33 cases.
Conclusions
Thyroid cancer in Albania like in all over the world is increasing. There is a female predominance 77.5% of thyroid cancer. Papillary type is the predominant form 52.9% but the follicular type has a high prevalence 32.7%, may be these is related with iodine deficiency which predominate in our country. The most frequen presurgical diagnoses was multinodular goitre. In 2007, we had 4 new cases ages 13 years old.

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The influence of radioiodine therapy on some parameters of oxidant/antioxidant balance in patients with subclinical hyperthyroidism

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Oxidative stress plays an important role in hyperthyroidism induced – tissue damage.
We aimed to determine whether radioiodine therapy (RIT) has benefit effect on the oxidant and antioxidant status in subclinical hyperthyroidism.

Material and methods
We studied 40 patients with untreated subclinical hyperthyroidism, (33 female, 7 male), aged 23–78 years; 14 patients with multinodular goitre, and 25 patients with solitary autonomous nodule. Twelve normal adult volunteers as control group.
Before the treatment all the patients has normal levels of serum fT3, fT4, low levels of serum TSH (<0.1 mU/l) and effective half-life was more than 3 days. Malignant changes were excluded in all nodules by fine needle aspiration biopsy.
In the investigated groups, we evaluate malondialdehyde (MDA) as a marker of oxidative stress, glutathione (GSH) and glutathione peroxidase (GPx) activity as a parameters of antioxidant system before and 6 months after RIT.
The serum fT4, fT3 and TSH were evaluated before and monthly up to 12 months after RIT. Thyroid ultrasound, and thyroid scan were done before and after12 months of ^{131}I therapy. The activity dose was calculated by Marinelli's formula and ranged between 200 and 600 MBq. The absorbed dose ranged between 160 and 280 Gy.
Results
Subclinical hyperthyroidism caused a significant increase in MDA level ($P<0.05$) as well as a significant decrease in GPx activities ($P<0.05$) and GSH level ($P<0.05$) compared to euthyroid controls subject.
Achievement of euthyroidism after 6 months of radioiodine administration resulted in a significant decrease of MDA, significant increase of GSH and non significant increase in GPx activities. Thyroid volume reduced to 45% (average); 38 patient were in euthyroidism, two patients received 2 doses of RIT.
Conclusions
Our results confirm the imbalance of the antioxidant/oxidant status in subclinical hyperthyroidism. RIT was more effective to improve these balances.

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Thyroid volume, selenium levels and nutritional habits in Southwestern Albania

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Introduction
Aim: it is known that environmental factors are involved in the development of goiter. A high number of goitrous patients was identified among patients attending the general medicine outpatients' in a mountainous region in Albania. We examined possible associations of thyroid enlargement with nutritional factors.
Methods
One hundred and twelve consecutive patients (mean age 52.8 ± 12.1, 104 female) attending the outpatients of the General Military Hospital of Gyrocaster, Southwestern Albania, who either took thyroxine ($n=27$) or were suspected to have thyroid disease based on symptoms and physical examination were studied. Thyroid parameters and selenium levels were determined. The type and frequency of food consumption was recorded; thyroid ultrasound was performed.
Results
Thyroid volume was above median (20.35 ml) in 51% of patients. Thyroid volume correlated negatively with the frequency of lamb and goat meat and vegetables consumption ($P=0.01$). Mean thyroid volume was significantly lower in those eating lamb or goat >1 times a week (21.4 ± 13.3 vs 31.9 ± 23, $P<0.01$). There was no association between current selenium levels and thyroid volume. All consumed food was home produced. The association of thyroid volume with lamb meat consumption was independent of sex, educational status or occupation ($P=0.004$, multivariate analysis). Forty-three percent had TSH <0.3 μU/ml (those on thyroxine were excluded). In this group, log TSH correlated negatively with thyroid volume and fT4 levels ($P=0.008$), indicating the presence of autonomy (TSHRab found positive in two subjects).
Conclusions
Nutritional factors appear to be involved in the development of goiter in Southwestern Albania. No role of selenium deficiency was found. The higher consumption of lamb and goat meat and vegetables, all non-industrialized, appears to be protective. One cannot exclude the possibility that this finding reflects better socioeconomic status, although this was not identified. Unrecognized subclinical hyperthyroidism probably due to thyroid autonomy is quite common.

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Case report: a case of diffuse large B-cell lymphoma presented by instantaneous enlargement in a known thyroid nodule and diagnosed by fine needle aspiration biopsy

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Primary thyroid lymphoma is an uncommon malignancy; it accounts for $<2\%$ of all extranodal lymphomas and $<5\%$ of all thyroid malignancies.
Case report
A 52-year-old male patient who was known to have a nodule in his thyroid gland for many years presented with instantaneous enlargement of his existing nodule and with appearance of ipsilateral cervical lymphadenopathy. On thyroid ultrasonography, a giant nodule of $38.4\times31.6\times45.5$ mm size, and containing macro and microcalcifical areas was detected in the left lobe. In the ipsilateral anterior cervical region, a lymphadenopathy with ultrasonographically malign appearance, in size of $23.4\times15.6\times32$ mm was observed. The patient was euthyroid in laboratory findings and his thyroglobulin and calcitonin values were in normal ranges. As cytomorphological appearance was consistent with anaplastic carcinoma in ultrasonography-guided aspiration biopsies of thyroid nodule and lymphadenopathy, immunohistochemical staining was performed for differential diagnosis of lymphoma. Diffuse large B-cell lymphoma cells showing CD20 involvement were seen in cytological specimens. The histological specimens of the patient who underwent total thyroidectomy and cervical neck dissection were examined. While normal thyrocytes were detected in thyroid gland, atypical lymphoid cells showing diffuse infiltration in thyroid tissue were observed in the nodule, which were stained diffuse, strongly positive for CD20 and BcL and negative for CD45 RO. In the lymph node, diffusely infiltrating atypical lymphoid cells with similar immunohistochemical staining properties were observed. According to Revised European–American Classification of Lymphoid Neoplasms, the diagnosis was diffuse large B-cell lymphoma. The patient was accepted as stage I-EB. Rituximab, Doxorubucin, cyclophosphamide, vinciristine, and prednisone chemotherapy was started. We introduce the case in order to emphasize that infiltration of diffuse large B-cell lymphoma should be recalled with instantaneous enlargement of nodules known to be benign previously, and when cytological specimen is uncertain, immunohistochemical staining should be performed for differential diagnosis.

P763

Case report: percutaneous laser ablation (PLA) to a functional euthyroid autonomous thyroid nodule, and histopathologic effects of PLA on nodule after thyroidectomy at 2nd year of procedure

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Objective
Percutaneous laser ablation (PLA) is a new procedure utilized to decrease nodule volume in symptomatic thyroid nodules, to provide euthyroidism in autonomous hyperfunctioning nodules, and to decrease compressive symptoms palliatively in malignant nodules. Standard treatment in autonomous thyroid nodules is

radioiodine and surgery. In this report, we aimed to present this case to demonstrate changes in nodule histopathology, which was undergone thyroidectomy two years after PLA procedure.
Case report
We applied PLA in a 25-year-old woman with euthyroid solitary autonomous thyroid nodule due to compression symptoms. A total of 3000 J of energy was applied in 600 s with a power of 5 W in single session. Initial nodule volume was 13.50 ml, which regressed to 4.20 ml within 6 months. However, nodule size began to grow at 9th month, and became 6.50 ml in 1 year. Nodule volume increased to 10 ml at second year of procedure, and compression symptoms reappeared. Thyroidectomy was performed two years after PLA. Macroscopically, thyroidectomy material revealed a white–gray area at the procedure site. Microscopically, there was minimal fibrosis, scarce amounts of old hemorrhagic findings, and multiple microfollicular structures containing colloid in their lumen. Lymphocyte infiltration was observed in thyroid tissue surrounding the nodule.
Conclusion
We did not observe any histopathologic effect of laser on thyroid tissue except lymphoid infiltration. This is the most important histopathologic finding indicating that PLA procedure was a minimally invasive and reliable technique. These are the first long-term data of histopathologic effects of PLA in a benign thyroid nodule which tended to re-grow after procedure. According to these data, PLA application on thyroid nodules is considered safe in long-term.

P764

Clinical features and outcome of papillary thyroid cancers <2 cm: univariate and multivariate analyses

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An increasing prevalence of papillary thyroid carcinomas (PTC) of small size have been recorded in recent years. These tumors have a favorable outcome in most cases, and total thyroidectomy without radioiodine therapy is suggested for microcarcinomas (defined as ≤ 1 cm). The 6th edition of the TNM staging system for thyroid cancers includes in the T1 category tumors ≤ 2 cm, limited to the thyroid. Aim of the present study was to evaluate the clinical/pathological features and the outcome of 251 patients with PTC dividing them into 2 groups according to tumor size (A: ≤ 1 cm, $n=156$; B: $>1 \leq 2$ cm, $n=95$). No differences between the 2 groups were observed in the mean follow-up (70 ± 44.2 and 66 ± 44.2 months), in the age at the diagnosis (47.5 ± 13.7 and 44.7 ± 16.2), in the gender (female in 68 and 61% of cases, respectively) and in the multicentricity (58 and 54%). A higher prevalence of incidentally discovered carcinomas was found in group A compared to group B ($P<0.0001$), while lymphnode metastases and extracapsular invasion at surgery were significant more frequent in group B ($P=0.02$ and $P<0.0001$, respectively). Concerning the outcome, no significant differences were found between the 2 groups, and in particular disease remission was observed in 90% of patients of group A and 84% of patients of group B. When multivariate analysis was applied to all tumors smaller than 2 cm, disease persistence/recurrence resulted to be associated with multicentricity, lymphnode metastases at diagnosis and extracapsular invasion ($P=0.02$, $P=0.01$, $P=0.04$, respectively), but not with tumor size.
In conclusion, T1N0 unicentric tumors has a favorable outcome independently from the tumor size (≤ 1 cm or >1 and ≤ 2 cm) and should be treated with a non-aggressive approach, avoiding radioiodine ablation.

P765

A prospective study on a large series of non toxic multinodular goiters treated with radioiodine: results at long term follow-up

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Radioiodine (^{131}I) administration has been found to be effective in the treatment of hyperfunctioning and normofunctioning multinodular goiter. Moreover, lithium therapy has been shown to enhance the radioiodine efficacy and to reduce the transient thyrotoxicosis induced by radioiodine.
Aim of the present study was to evaluate the long term efficacy of ^{131}I with or without lithium administration in the treatment of non toxic multinodular goiter (NTMG). Eighty patients without thyroid auto-antibodies were randomized into two groups: # 1, treated with con $I^{131}+$ lithium (900 mg/die for 6 days) and # 2, treated with ^{131}I alone. The ^{131}I activity (MBq) was calculated according to thyroid volume (TV) (222–600 MBq, with a mean dose/patient of 555 MBq). Ultrasonography and biochemical analyses were evaluated before and 1, 3, 6, 12 and 24 months after ^{131}I. A mean thyroid volume reduction of 49% was observed at two years of follow-up without significant differences between the two groups. Forty-five patients were re-evaluated after a longer follow-up (41–70 months). Thyroid volume further decreased (mean reduction: 50.7%) in 61% of these patients, and remained unmodified in the remaining cases, without significant differences between the two groups. A permanent hypothyroidism was diagnosed in 21.4% of patients during the first two years of follow-up, without differences between the 2 groups; no additional patients developed hypothyroidism in the following 3 years of follow-up.
In conclusion, present data confirm the efficacy of ^{131}I in the treatment of non toxic goiter. The maximum thyroid volume reduction was achieved 2 years after the therapeutic administration and no relapse was observed in the following years. Permanent hypothyroidism developed in about 20% of cases during the first 2 years of follow-up. The association with lithium treatment neither enhanced the efficacy to ^{131}I treatment nor influenced the development of permanent hypothyroidism.

P766

Higher prevalence of nodular goiter and thyroid carcinoma in patients with familial adenomatous poliposis (FAP)

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FAP is a dominant autosomic syndrome, due to mutation of the APC oncosuppressor gene, characterised by adenomatous polyps at the colon–rectal level, that progres to malignant degeneration. FAP extraintestinal manifestations include, although rare, the thyroid nodules and tumours. Neck ultrasound (US) is routinely useful to identify thyroid nodules, but the characterisation of their nature requires the cytology by a FNAb. The exact incidence of the thyroid tumours on FAP syndrome is unknown.
Aim
To evaluate the prevalence of thyroid nodules and tumours in a group of FAP patients.
Subjects and methods
Forty-six consecutive FAP outpatients (25♀–54%, 21 ♂–46%; mean age $46.76 \pm$ DS 13.96 years) underwent neck US. The lesions above 5 mm of diameter underwent FNAb. The control group was of 599 subjects who underwent FNAb because of thyroid nodules.
Results
From the 41 FAP patients included in the study, 18 (43.9%) (13♀, 5♂) showed thyroid nodules. The FNAb was performed on 12 FAP patients with thyroid nodules (66%; 8♀, 4♂): 2♀ (16.67%; mean age 34.05 years) had a diagnosis of papillary thyroid carcinoma. From the 599 FNAb of the control group 23 (3.8%) had a diagnosis of thyroid carcinoma (6♂-1% and 17♀-2.8%; mean age $48.88 \pm$ DS 14.83 years).
Conclusion
The nodular goiter is very common in FAP (43.9%). Furthermore, cytology at FNAb highlights as the prevalence of thyroid tumours is higher in FAP group (16.67%), also when compared with a control group, selected from a iodine deficient area with an high incidence for thyroid carcinoma (3.8%). These data show a possible correlation between the two diseases and suggest the need for a careful follow-up of thyroid lesions in patients with FAP, by means of both neck US and FNAb.

P767

Case report: subcapsular hematoma complication during percutaneous laser ablation (PLA) to a hypoactive benign solitary thyroid nodule, and literature review of PLA- related complications

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Objective
Ultrasound (US) guided percutaneous laser ablation (PLA) is a new procedure utilized to decrease nodule volume in symptomatic thyroid nodules, to provide euthyroidism in autonomous hyperfunctioning thyroid nodules, and to decrease compressive symptoms palliatively in malignant nodules. Standard treatment protocol in symptomatic benign hypoactive thyroid nodules is surgery, if needed LT4 suppression, and radioiodine treatment in certain applications. We applied PLA to 42-year-old male patient with 16.8 ml volume solid hypofunctioning benign thyroid nodule.
Case report
The procedure was planned to inferior and superior parts of the nodule. First application to inferior part of nodule was performed in single session with 3 W power, and 720 J was applied in 240 s. Control US images after the procedure showed formation of subcapsular hematoma in inferior anterior part of nodule. Although patient had no complaints like dysphagia, dyspnea, or pain, 2nd part of procedure was cancelled. Patient was closely monitored in coordination with surgical team. Hematoma was absorbed largely within 48 h. It disappeared completely at first month.
Conclusion
Various complications have been reported after diagnostic and therapeutic interventions to thyroid nodules. Cervical pain, hoarseness due to laryngeal injury, and hematoma are most frequent of these. PLA is applied as one of non-surgical treatment methods in treatment of solid thyroid nodules in recent years. Pain, hoarseness, fever, and hyperthyroidism have been reported as complications after PLA in several case series, but hematoma has not been, up to now, reported as a complication of PLA. We considered publication of this case report because development of subcapsular hematoma after PLA was not previously reported in literature.

P768

Thyroid size and thyroid function in children from iodine deficiency regions

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Background and aims
Diffused endemic goiter (DEG) development is not always explained by increased TSH levels. Most DEG patients (pts) have normal TSH concentrations, with tendency to decreased T4 and normal or slightly elevated T3. In the present study levels of thyroid hormones (TH) in pts with various degrees of thyroid size (TS) and weak iodine deficiency (ID) were analyzed.
Material and methods
In total DEG 137pts, aged 2–16 years were studied: 100 girls (g) (73%), 37 boys (b) (27%). TH-T3, T4, TSH were measured. Results were compared using Student's criterion (*t*-test). For each pt ultrasound was performed and antithyroid antibodies (TPO-Ab, Tg-Ab) were measured. For palpation assessment of TS WHO classification (1980) was used. TS ultrasound data were compared to upper volume limit (ml) in iodine sufficient areas (WHO-IDD Control International Council, 1997). Thyroid volume increase expressed as percentage and palpation results were compared and summarized: IA–increase from 0 to 10%, IB – from 10 to 50%, II – from 50 to 100%, III – above 100% increase.
Results
Hashimoto thyroiditis was excluded based on TPO-Ab, Tg-Ab. Sixteen pts, (g12/b4) with hypothyroidism (3 pts) and subclinical hypothyroidism (13 pts) were excluded T3 levels compared in pts with IB and III TS, were evidently higher in pts with III TS (2.43 ± 0.12 and 3.18 ± 0.14 nmol/l, $P < 0.001$). Significant difference in T3 levels between IB, II TS was not observed. Besides there was no significant difference in T4 levels while comparing various TS. TSH was evidently higher in pts with large goiter both in IB – II and IB – III TS (2.13 ± 0.2 vs 3.59 ± 0.54 mUI/ml, $P < 0.05$ and 2.13 ± 0.2 vs 3.49 ± 0.35 mUI/ml, $P < 0.001$).
Conclusions
T3 increase in ID areas gives adaptation possibility. Increased sensitivity to TSH may occur when ID is present. This explains relatively high, though normal, TSH levels in pts with large goiter.

P769

The influence of chronic GH excess upon thyroid gland morphology evaluated by ultrasound

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It is known that thyroid pathology is generally more frequently found in women than in men. The somatotroph axis stimulates skeleton growth, but also the development of smooth tissues, including the thyroid gland. We investigated the effect of chronic GH excess upon ultrasound-evaluated (7.5 MHz linear probe) thyroid gland morphology of 74 patients (40 women şi 34 men) diagnosed with acromegaly. When compared to mean thyroid volume of age- and sex-matched non-acromegalic controls, mean thyroid volume was found to be significantly increased in both male and female acromegalic patients (24 ± 8.8 vs 13.2 ± 6.1 ml for males, 23.4 ± 10.2 vs 10.7 ± 7.9 ml for females and 23.9 ± 8.9 vs 11.1 ± 3.2 ml for the whole group, $P < 0.05$ at *t*-test). The incidence of goiter (defined as thyroid volume of over 20 ml in females and over 25 ml in males) and of thyroid nodules was higher in female than in male controls (5.5 vs 2.4% for goiter and 13 vs 4% for nodules, $P < 0.05$ at the χ^2 test). Goiter incidence was much higher in acromegalic patients (56% in acromegalic women vs 5.5% in healthy age and sex matched controls and of 43% in acromegalic men vs 2.4% in healthy controls, $P < 0.0001$). The incidence of thyroid nodules was again high both in acromegalic females (40 vs 13% in controls) and males (33 vs 4% in controls, $P < 0.005$). No correlation between GH levels and thyroid volume or nodular volume was found. IGF-1 was assessed only in a minority of our study group. The only parameter correlated to the thyroid volume and to the incidence of thyroid nodules observed in our group was the length of disease evolution ($P < 0.05$). We did not notice the appearance of thyroid neoplasias or of nodular autonomy in our patients. Chronic GH excess increases thyroid volume and nodule incidence both in women and men, attenuating sex differences in thyroid morphology.

P770

New mutations demonstrate intracellular iodine retention in Pendred syndrome

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Pendred syndrome is an autosomal recessive disorder with congenital-sensorineural deafness and goiter due to mutations in *SLC26A4*, that encodes a transmembrane protein, Pendrin. In thyrocytes, Pendrin is proposed to act at the apical pole to transport intracellular iodide into the follicular lumen.
A Galician Pendred compound heterozygous patient was studied; a c.297delT in exon 3 and a new splicing-mutation c.416-1G<A were found, introducing premature stop codons in the protein.
A thyrocyte primary cell-line, T-PS2, was obtained from the patient and compared with primary thyroid lines from our BANTTIC (Bravo *Oncogene* 2003, Bravo *Clin Cancer Res* 2005). NT (normal thyrocytes) and T-PS2 have similar epithelial appearance, with follicle-like structures and expressed thyroglobulin.
By western-blot, NT and T-PS2 showed similar levels of plasma-membrane NIS (Na^+/I^- symporter), while only NT showed high levels of plasma-membrane pendrin. Confocal immunofluorescence localized Pendrin in NT at a spot near the nucleus, Golgi location, and in narrow lines typical of plasma-membrane localization. Opposite, in T-PS2 Pendrin positivity was located exclusively in Golgi, indicating retention of truncated proteins.
Iodine-uptake measurements were performed. Steady-state uptake was 3-times higher in T-PS2 than in NT. Time-course uptake in NT showed a fast uptake followed by a plateau after 5 min onward; T-PS2 showed a progressive increase in iodide level till 30 min; V_{max} was twice as high in T-PS2.

Efflux was fast for NT: at 15 min all radioactivity had effluxed while 40% still remained intracellularly in T-PS2.
We used dose–response curves to study Michaelis-Menten iodine-uptake kinetics. After 5 min, T-PS2 had already reached a Km similar to that described for NIS at equilibrium (22 ± 4.8 μM). Opposite, NT only achieved equilibrium after 1 h of incubation.
In summary, normal thyrocytes behave as a complex system with two opposite transporters (NIS and Pendrin) that reach equilibrium slowly. Pendred thyrocytes accumulate iodine through NIS, although iodine leaves the cell inefficiently through other non-specific transporters.

P771

Thyroid hormone replacement using a combination of levothyroxine plus slow-release liothyronine: beyond proof of principle

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Objective
To assess if adding slow-release liothyronine (SR-T_3) to levothyroxine (l-T_4) reduces persistent hypothyroid symptoms and signs on replacement with l-T_4 alone.
Design
Open label, two-period cross-over, cohort comparison. The main outcomes were changes in the serum T_3 concentration and residual clinical hypothyroid indicants. Each study period was 10 weeks. The sample size required was determined to be 9 patients.
Subjects
Three men and 15 women, aged 31 to 72, with primary, autoimmune hypothyroidism who had persistent hypothyroid indicants in spite of normal or suppressed thyroid stimulating hormone (TSH) levels on l-T_4 alone entered the study after giving informed consent.
Methods
Subjective clinical indicants, scored as ordinals from 0 to 3, and objective clinical and biochemical indicants measured on a numerical scale, were compared during treatment with l-T_4 alone to treatment with l-T_4 *plus* SR-T_3 titrated to an increase in the serum T_3 of 0.50 pmol/l.
Main outcome
Adding an average of 13 (range 5–20) μg per day of SR-T_3 elevated the serum T_3 by a significant average (95% CI) of 0.80 (0.60–1.00) pmol/l. The mean clinical hypothyroid indicant score (95% CI) of 13.9 (10.8–17) on monotherapy, fell by a significant average of 10.8 (9.0–12.6) (84 (70.3–97.3) percent) to 3.3 (0.9–5.5). The T_4/T_3 ratio fell significantly from 4.0 (3.7–4.3) to 3.5 (3.1–3.7). There were no significant changes in the initial mean l-T_4 dose of 88 (74–102), or mean TSH of 1.5 (0.9–2.1) mU/l and T_4 of 16.3 (15.2–17.4) pmol/l. No patient exhibited evidence of over-replacement.
Conclusion
Treating primary, autoimmune hypothyroid patients with persistent hypothyroid symptoms and signs using a combination of l-T_4 plus SR-T_3 resulted in a significant rise in the serum T_3 level and decrease in the persistent hypothyroid indicants.

P772

Usefulness of thyroid echographic pattern description: predictive value at onset and in the evolution of thyroid diseases

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Objectives
The study of the real indications for thyroid ultrasound and in which pathological conditions the echography is of real use and not for abuse.
Material and methods
In the last 10 years, over 13 000 echographies were performed in 5136 consecutive patients for 'thyroid disturbances'. In a previous paper we described 7 echographic patterns for thyroid ultrasound (9th ECE). The diagnosis of Hashimoto's thyroiditis (HT) was considered if antithyroperosidase antibodies (ATPO) were above 34 IU/ml. Idiopathic myxedema (IM) was considered if hypothyroidism was not associated with HT. Graves–Basedow disease (GBD) was based on positive TSH receptor antibodies (TRAB).
Results
Diagnostic at onset: normal thyroid was found in 3489 cases, HT in 487 cases, and GBD in 94 cases from whom 65% had an overlap between ATPO and TRAB. Subacute thyroiditis occurred in 13 cases, IM in 34 cases, benign nodules in 1009 patients and malign nodules in 10 cases. The echographic description at onset revealed a high positive predictive value (PPV) of 92% for pattern 1 in the diagnosis of HT. The high negative predictive value for pattern 4 (82.73%) suggests that a macronodule is rarely associated with HT. In GBD, pattern 5 had a medium PPV (42%). Changing echographic pattern in evolution suggested that in HT, the pattern changed in only 12 patients (2.5%). In subacute thyroiditis, the echographic shape changed suddenly from day to day. In GBD, in 24 patients without Hashimoto association (75%), the echographic shape changed from pattern 5 to quasi-normal (pattern 7), after 3–5 years. In IM, no change was registered. Only 25 benign nodules (2.5%) improved and changed their shapes. No changes were observed for malign nodules during follow-up prior to surgery (1–6 months).
Conclusions
It seems that thyroid echography is performed 3–4 times more than needed.

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Surprising: hyperlipidemia in subclinical hyperthyroidism!

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Subclinical hyperthyroidism is a biochemical diagnosis defined with suppressed levels of TSH and normal levels of thyroid hormones. One of the main metabolic responses to thyroid hyperfunction is peripheral lipolysis and release of free fatty acids (FFA). The aim of this study was to compare levels of cholesterol, LDL, HDL and triglycerides in persons with subclinical hyperthyroidism to age and BMI matched euthyroid healthy control. We evaluated 45 persons in two groups: 1st group: 30 patients (26 women and 4 men) with subclinical hyperthyroidism, mean age 49.13 ± 10.21 years, mean BMI 25.18 ± 4.04 kg/m^2, with laboratory proven subclinical hyperthyroidism. Second group: 15 healthy euthyroid controls, mean age 49.40 ± 9.84 years, mean BMI 23.74 ± 4.09 kg/m^2. None of them had a history of coronary heart disease, signs of liver and/or renal dysfunction or were previously treated with lipid lowering drugs. Statistical analysis was performed with t-test. In the 1st group, mean cholesterol level was 6.15 ± 1.10 mmol/l, mean LDL level was 4.08 ± 0.96 mmol/l, mean HDL level was 1.46 ± 0.49 mmol/l and mean triglycerides level was 1.71 ± 0.57 mmol/l. In the 2nd group, mean cholesterol level was 5.38 ± 1.03 mmol/l, mean LDL was 3.16 ± 0.68 mmol/l, mean HDL level was 1.37 ± 0.37 mmol/l and mean triglycerides level was 1.29 ± 0.55 mmol/l. There was a significant difference in levels of cholesterol ($P < 0.05$; $P = 0.035$) and triglycerides ($P < 0.05$; $P = 0.0034$) and the difference in LDL was high ($P < 0.01$; $P = 0.005$). There was no difference in HDL levels ($P > 0.05$; $P = 0.55$). The clinical presentation of subclinical hyperthyroidism is quite subtle which points to not so high metabolic rate of this condition and no real metabolic need for utilization of released FFA's. This may be the possible explanation for unexpected and surprising result of hyperlipidemia in subclinical hyperthyroidism.

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The variation intercellular adhesion molecule-1 level in primer and postoperative hypothyroid patients with treatment

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Background and aims
It is reported that intracellular adhesion molecule-1 (ICAM-1) is important in autoimmune thyroid diseases also it is an important point that ICAM-1 level is high in postoperative hypothyroid cases. In literature, it is said that the level of plasma soluble ICAM-1 can be used as a evidence immune system activation and inflammation, and also it can be used for the diagnosis of some inflammatory and autoimmune diseases. The aim of our was to compare the level of ICAM-1 in hypothyroid cases who have different ethiologic origins and was to investigate the among treatment variation.
Material and methods
Sixteen primer hypothyroidism, 16 postoperative hypothyroidism, totally 32 female patients were chosen for the study and also 16 healthy, no past and familiar disease were chosen as a control group. The patients who have type 2 diabetes, who have inflammatory and infectious disease and smokers were left from this group when it's diagnosed in patients and control groups and when the level of TSH is normal biochemical fast parameters and level of ICAM-1 was calculated.

Results
Primer hypothyroid, postoperative hypothyroid and control groups were approximately similar for age (38.6±7.3, 38.0±7.3, 40.6±6.3). And also body mass index was similar in patient and control groups the level of beginning ICAM-1 level in postoperative hypothyroid patients was found higher than control group (46.6±10.7, 27.1±2.3, $P<0.001$). When two hypothyroid group were compared the level of ICAM-1 was found really high in primer hypothyroid group (46.6± 10.7, 39.4±5.1, $P<0.05$). In both groups, the level of euthyroid ICAM-1 was found very high than the beginning datas (46.6±10.7, 51.3±10.5, $P<0.001$, 39.4±5.1, 45.3±6.8, $P<0.001$).
Conclusion
ICAM-1 level can be used for investigating hypothyroid cases. The level of ICAM-1 is increasing in hypothyroid cases with the treatment. The level of ICAM-1 can be used as a effectiveness parametria in hypothyroid cases.

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Tuberculosis of the thyroid gland: the chameleon of thyroid pathology
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Tuberculous thyroiditis is a very rare disease. Its clinical features are non-specific in most of the cases, mimicking other thyroid diseases. Confusions most frequently made are those with thyroid cancer and toxic nodular goiter. Therefore, diagnosis often comes as a histopathological surprise, after thyroid ablation for other reasons. Thyroid tuberculosis was diagnosed histopathologically in six out of 1232 cases of thyroid surgery. Preoperatory diagnosis was of toxic nodular goiter in two cases, and of thyroid carcinoma in the other four. We diagnosed other two cases of thyroid tuberculosis out of 2291 nodular goiters investigated by FNAB. The two patients were initially suspected of thyroid carcinoma and acute bacterial thyroiditis, respectively. Axillary lymph node biopsy in the first case showed the presence of giant epithelioid cell granulomas, with evolution toward caseous necrosis. Signs of inflammation accompanied by caseation were found in thyroid aspirates in both subjects. Löwenstein culture allowed the isolation of mycobacterium tuberculosis. Fine needle aspiration biopsy allows establishing the diagnosis before surgical intervention. Presence of epithelioid granulomas with necrosis and of acid-fast bacilli in the aspirate is very specific. When central caseous necrosis is present, the thyroid lesion is highly suspectable of being tuberculous even in the absence of cellularity. Further culture of the biopsy product on special media is then mandatory. Preoperatory diagnosis of thyroid tuberculosis may modify the therapeutic attitude.

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Is thyroid tuberculosis actually not rare?
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Background and aim
Thyroid gland is considered to be rare site of extrapulmonary tuberculosis (tbc), even in countries with a high prevalance. The aim of this study is to ases the correlation between thyroid nodules and pulmonary tbc with fine needle aspiration cytology (FNAC) and to find out the incidence of thyroid gland tbc.
Material and methods
The main purpose of this study is to find out tbc incidence in pulmonary tbc patients with thyroid nodules. FNAC is a rapid and minimally invasive approach to diagnose extrapulmonary tbc and has been used successfully in thyroid malignancies. Thyroid function tests and ultrasonography (USG) investigations were carried out an each patient. FNAC is carried out patients who had solid or dominant nodule in multinodular ones via USG.
Results
The present study included 596 cases of pulmonary tbc of which 201 cases had solid or multible nodules. Majority of 201 of 596 patients were in the age group of 18–72. Mean age was 40.16 with male to female ratio of 1:1.093. Except one, none of the patients diagnosed n tbc in thyroid nodule.
Conclusion
The incidence of tbc increasing worldwide, the unusual presentation of extrapulmonary tbc presents a diagnostic challenge. The findings of this study suggest that FNAC no need to be used as routine line of investigation fort he diagnosis of thyroid nodules in cases of pulmonary tbc.

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Hypothyroidism due to primary hypotriiodothyronaemia: a case report
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Objective
Report of hypothyroidism due to primary hypotriiodothyronaemia caused by a deficiency in peripheral generation.
Design
Case report and computer modelling.
Main outcome
A 28-year-old woman presented with hypothyroid symptoms and delayed ankle reflex relaxation times. She was not on any medications. The average thyroid stimulating hormone (TSH) concentrations was 2.5 (*N*: 3.8–5.5 mU/l), free thyroxine (T4) 12.3 (*N*: 10.5–20 pmol/l) and free triiodothyronine (T3) 3.15 (*N*: 3.50–6.50 pmol/l). Pituitary and hypothalamic hypothyroidism and secondary causes of the low T3 syndrome were ruled out. The reverse triiodothyronine (rT3) was 180 (*N*: 120–540 pmol/l). Serum selenium and iodine levels were normal. The log/linear plot of the TSH versus T4 values and suppression of TSH to sub-physiologic levels at mid-normal T4 concentrations, suggested a hyper-sensitive TSH-negative feedback mechanism. Computer modelling of the initial thyroid hormone levels and their response to L-thyroxine replacement was most compatible with a 30% primary decrease in peripheral type 2 deiodinase activity, and a 2% compensatory decrease in T3 inactivation, occurring in the presence of an extra sensitive TSH-negative feedback mechanism.
Conclusion
The patient's hypothyroidism was due to a deficiency in peripheral T3 generation caused by a primary deficiency in type 2 deiodinase activity, most likely as the result of a mutation in the gene coding for the enzyme.

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Hashimoto's encephalopathy: a rare cause of status epilepticus
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Background
Hashimoto's encephalopathy is a rare steroid-responsive condition associated with high antithyroid antibody titres. We report a case of autoimmune thyroiditis presenting with status epileptics.
Case
A 36-year-old non-epileptic Caucasian lady presented as an emergency with uncontrolled generalised seizures requiring sedation, short-term ventilation and maintenance sodium valproate therapy. Further enquiry revealed a 3–4 week history of personality change, loss of memory and easy fatiguability, and a 1 week history of facial twitching. She had no previous history to suggest an underlying tendency to seizures and her past medical history was unremarkable. There was a family history of autoimmune thyroid disease. Physical examination was normal but higher mental functions were significantly impaired (initial Addenbrooke's cognitive examination (ACE) score 74/100).
Investigations revealed normal full blood count, clotting, ESR, liver and renal function, calcium and glucose. Anti-nuclear antibodies were detectable at 1:100 but anti-dsDNA, ENA and ANCA were all negative. CT scan of the head was normal as was a subsequent MRI including a T1 coronal volume sequence. The EEG showed no focal abnormalities. Cerebrospinal fluid protein was marginally raised but no organisms were seen and culture was negative. Anti-voltage gated potassium channel antibodies and anti-neuronal antibodies were negative. Thyroid function tests showed normal FT4 of 15.8 pmol/l and raised TSH of 30.23 mU/l; anti-TPO antibodies were markedly elevated (>1300 kU/l; normal <60). She was treated with thyroxine replacement, pulsed methylprednisolone and maintenance high-dose oral prednisolone. Her memory improved markedly with a rise in ACE score to 86/100 at discharge.
Discussion
Although seizures are a well-recognised feature of Hashimoto's encephalopathy, it is rarely considered in the differential diagnosis of epilepsy. Our case illustrates the importance of thyroid function assessment in patients with unexplained status epilepticus and reinforces the value of high dose corticosteroid therapy in the treatment of Hashimoto's encephalopathy.

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Thyroid function is associated with insulin sensibility

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Thyroid disease is associated with atherosclerosis cardiovascular disease. This is undoubted for overt hypothyroidism, but is controversy in subclinical hypothyroidism hittle is known about the relationship between insulin resistance and thyroid function. It is well known that insulin resistance is the main part of metabolic syndrome. We aimed to investigate the relationship between thyroid function, body mass index (BMI) and homeostasis model assessment index for insulin resistance (HOMA-IR) in patients with heart ischemic disease.
Methods
Seventy-three patients were included in our study. Heart ischemic disease was confirmed by the results of coronarography. We studied age, gender and BMI of our patients. Fasting blood samples were taken for measuring of TSH level, glucose and insulin concentration. HOMA-IR was calculated as fasting insulin (mU/l) times fasting glucose (mmol/l) divided by 22.5. Patients with diabetes were excluded from the study.
Results
Middle age of our patients was 57.23 ± 0.48 years, BMI was 28.23 ± 230.19 kg/m^2 and TSH level was 2.61 ± 0.74 IU/l. In accordance with BMI all patients were divided in 3 groups: I – normal weight, II – weight abudance, III – obesity. TSH level was reliable higher in patients with obesity (3.05 ± 1.14 IU/l) and weight abundance (2.73 ± 0.26 IU/l) than in group with normal weight (1.52 ± 0.34 IU/l) ($P = 0.02$ and 0.01). Age was nearly the same in all groups. HOMA-IR was the highest in the group of obesity (4.67 ± 1.12). In group I and II, HOMA-IR was statistically lower: 1.30 ± 0.26 and 1.52 ± 0.74 ($P = 0.002$ and 0.05).
Conclusion
In patients suffering from heart ischemic disease and obesity, TSH level and HOMA-IR index were statistically higher than in patients with normal weight. We can propose that thyroid hormones may participate in mechanisms of peripheral insulin resistance.

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Severity of coronary atherosclerosis in patients with different TSH levels

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Subclinical hypothyroidism (SH) is very frequent condition in older population. If can occur in 12–20% of patients older than 60. It is well known today that thyroid hormones can increase gene expression of APO-B receptors in the liver and by this way improve catabolism of atherogenic lipoproteins. They also can regulate processes of contractility and weakening of heart muscle. Decrease of cardiac output and diastolic disfunction of left ventricle can be revealed in SH patients. So even mild thyroid failure can promote the development and progression at heart ischemic disease. Results of coronarography (CG) can reliable reflect the severity of coronary atherosclerosis. It seems to us very important to compare results at CG with TSH level of heart ischemic disease patients.
Methods
Four hundred and eighty-nine patients participated in our study. In all patients, CG was performed by standart methodology of Judkins *et al.* We studied age, gender, body mass index of patients, their smoking history, genetic predisposition. Fasting blood samples were taken for measuring of TSH level by reagent of third generation.
Results
There were 77.4% of men and of men and 22.6% of women. Middle age was 56.53 ± 0.41 years. BMI was 27.23 ± 0.17 kg/m^2; TSH level was 2.53 ± 0.30 IU/l. In 9.5% of patients, SH was revealed (TSH level was more than 4.0 IU/l), in 5.8% of cases TSH level was <0.5 IU/l, which characterize. SH was revealed in 15.8% of women and 6.7% of men. There was positive correlation between TSH level and BMI in all patients ($P = 0.008$; $r = 0.127$) and TSH level and age in women ($P = 0.043$; $r = 0.143$). Multivessel damage of coronary vessels correlated with man gender, age, duration of smoking, genetic predisposition, hypertension and diabetes mellitus and TSH level more than IU/l ($P = 0.041$, $r = 0.172$). The trunkal damage of left coronary artery was also associated with elevation of TSH level. In patients with SH trunkal damage was revealed in 38% of cases, when in patients with normal TSH level only in 19.3%.
Conclusion
In heart ischemic disease patients, SH was associated with women gerder, elevation of BMI and severity of coronary atherosclerosis.

P781

Iodine status in pregnant women: do they have sufficient information about the importance of iodine nutrition during the pregnancy?

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The recommended nutrient intake (RNI) for iodine during pregnancy has been re-evaluated in 2005 by an international expert committee under the auspices of the World Health Organization (WHO). The consensus reached was that the RNI for iodine during pregnancy should range between 200 and 300 μ/d with an average of 250 μ/d.
To asses the adequacy of the iodine intake during pregnancy in a population, urinary iodine concentration (UIC) should be measured. UIC should ideally range between 150 and 250 μ/l.
Objectives
To identify the current state of iodine nutrition in pregnant women using UIC and to estimate the level of information about the importance of iodine requirements in pregnancy.
Patients and methods
We studied a cohort of 185 pregnant women, average age 31.7 ± 5.0 years, gestational age 26.9 ± 5.6 weeks, we asked them about the use of de iodized salt and supplements in the form of potassium iodine or multivitamin tablets specifically designed for pregnancy and too about their knowledgments related to the importance of iodine intake. We considered UIC adequate 150–250 μ/l, mild deficiency 160–100, moderate 100–50, severe <50, more than adequate 250–500 and excessive >500 μ/l.
Results
The median UIC was 143 μ/l. In 53.2% was <150 μ/l (28.5% mild, 23.1% moderate and 1.6% severe deficiency), 25.8% had an adequate UIC, 16.1% more than adequate and 4.8% excessive. About 74.2% ingested tablets, 56.5% used iodized salt. Only 21.5% had received information by health personal and 11.3% in published information. About 50% of pregnant women with UIC <150 μ/d didn't consume iodized salt ($P < 0.05$), 35% didn't ingested oral supplements ($P < 0.05$) and 87.6% hadn't received information ($P < 0.05$).
Conclusions
(1) Pregnant women studied had mild deficient UIC. (2) The proportion of pregnants that declare use oral supplements or idoized salt is elevate but in near 50% the state of iodine nutrition is insufficient. (3) The information about the importance of iodine nutrition during pregnancy is scarce.

P782

Is Graves' disease the most frequent immune association with Hashimoto thyroiditis?

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Aim
To re-evaluate the prevalence of autoimmune associations in Hashimoto's thyroiditis (HT).
Subjects and methods
The study was performed on 469 consecutive patients with HT and 536 without. The diagnosis of HT was based on an antithyroperoxydase antibodies (ATPO) level above 34 IU/ml. The subjects were evaluated for other associated immune diseases. The statistical analysis used the χ^2 test.
Results
Fifty-seven patients with HT ($\approx 12\%$) associated one or more immune diseases. The most important associations were: chronic hepatitis in 13 cases (22%), vitiligo in 9 patients (17%), Biermer anemia in 6 cases (11%), rheumatoid arthritis in 5 cases (9%), diabetes mellitus type I (IDDM) in 3 patients, immune vasculitis in 2 cases. In the control group 8.3% of cases had an immune disorder. These were mainly: 16 cases with rheumatoid arthritis, 7 with hepatitis, 4 vitiligo, 3 systemic lupus erythematosus, IDDM, bronchic asthma, 2 cases with vasculitis and psoriasis. χ^2 test (one degree of freedom) $=3.09$, $P = 0.079$. 60 patients with increased ATPO (with HT) presented also hyperthyroidism with Graves-Basedow's disease (GBD). Prevalence was about 25%. Immune associations (other than GBD) did not modify the echographic and functional patient phenotype.

Discussions
In our previous paper (9th ECE), we found a prevalence of immune associations in HT of 15.8% compared with 5.19% in the control group. Now, in the control group we found more immune diseases. If increased ATPO levels (acting through antibody-dependent cellular cytotoxicity) mean HT and increased thyroid stimulating immunoglobulins (acting at receptor level) mean GBD, then it is obvious that there could be two distinct concomitant immune thyroid diseases.
Conclusions
Contrary to those expected and already communicated, in HT immune associations seem not to be higher than in controls, but the most common immune association in HT is GBD.

P783

Effects of thyroid-stimulating hormone suppression with levothyroxine in reducing the volume and prevention the growth of solitary thyroid nodules

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Suppressive therapy with thyroxine is the standard conservative treatment for solitary benign thyroid nodules. However, factors which may influence the response to treatment remains controversal, as well as the risc of thyroxine administration.
We prospectively evaluated the effects of twelve months thyroid-stimulating hormone suppression with Levothyroxine (L-T4) in reducing the volume of benign solitary nodules, and the effects of reduced serum TSH level on both cardiovascular system and bone loss in postmenopausal women. All of 52 patients were prescribed individual Thyroxine in a dose to keep TSH levels 0.3–0.5 mU/l. Clinical, laboratory, ultrasographic, cytological features of the nodules, were assessed before treatment, in three months interval, and after 12 months. Bone mass density was measured before treatment and in one year interval in postmenopausal woman. Cardiovascular parameters (BP, heart rate), and ECG were monitoring during 1 year period. The therapy was effective in 25.6% persons. In 5.76% persons nodules showed evolution, and in 74.4% persons there was no significant change during 1 year period. In group of responders, the mean reduction of volumen of nodules was 19%, homolateral lobus 6.4% and contralateral 3.5%. The best answer on therapy showed younger persons with colloid nodules. There were no adverse cardiovascular manifestations during 1 year therapy. Subclinical hyperthyroidism in postmenopausal woman did not result in accelerated bone loss.
In conclusion, we found suppressive L-T4 therapy in selected patients can be effective in reducing solitary thyroid nodule volume, and there is no risk of the administration of thyroxine in 1 year period.

P784

Growth inhibitory actions of human proepidermal growth factor cytoplasmic domain (proEGFcyt) are mediated by the ubiquitin-proteasome system in human thyroid carcinoma cells

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The cytoplasmic domain of the membrane-anchored human EGF proform is encoded by exons 22–24 and the function of this proEGFcyt is largely unkown. Stable transfectants of the human thyroid carcinoma (Ca) cell line FTC-133 were generated over-expressing (a) proEGFcyt, (b) truncated peptide version encoded by exons 22 and 23 (proEGF22.23), and (c) a natural splice version with a deletion of exon 23 and 24 (proEGFdel23). ProEGFcyt and proEGF22.23 transfectants, but not proEGFdel23 or mock clones, demonstrated a significant reduction in growth rates. This coincided with a marked post-translational reduction in EGFR and ErbB2 protein levels in proEGFcyt and proEGF22.23 clones. Incubation of these transfectants with the proteasome inhibitors MG132 and lactacystin diminished the growth inhibitory effect of proEGFcyt and reversed the down-regulation of EGF receptor proteins suggesting an involvement of the ubiquitin-proteasome system. Microarray analysis of FTC-133-proEGFcyt clones revealed a strong downregulation of the ubiquitin C-terminal hydrolase-L1 (UCH-L1), thus, identifying proEGFcyt as a new regulator of this de-ubiquitinating enzyme. Silencing of UCH-L1 gene activity and the absence of UCH-L1 protein was exclusively observed in proEGFcyt and proEGF22.23 FTC-133 transfectants but absent in proEGFdel23 and mock controls implicating a possible involvement of exon23 encoded peptide of proEGFcyt. Westernblot analysis of total ubiquitinated protein revealed significantly increased levels of cellular ubiquitinated proteins in proEGFcyt and proEGF22.23 transfectants. Specific knockdown of UCH-L1-containing proEGFdel23 and mock clones resulted in reduced EGFR protein levels similar to proEGFcyt and this decrease in EGFR was prevented in the presence of MG132. In summary, we present evidence for a novel and unique mechanism for the growth inhibitory actions of proEGFcyt in human thyroid Ca cells. We identified proEGFcyt as a new regulator of the de-ubiquitinating enzyme UCH-L1 and demonstrate that proEGFcyt-mediated silencing of UCH-L1 causes the decrerase in EGFR likely as a result in post-translational hyperubiquitination of EGFR in human thyroid Ca cells. These findings may have important implications for the design of new treatments particularly of undifferentiated thyroid cancer.

P785

Isolated ACTH deficiency as a cause of impaired well-being in patients with primary hypothyroidism

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Objective
Isolated ACTH-deficiency (IAD) is considered a rare autoimmune endocrinopathy most frequently associated with autoimmune thyroid diseases (ATD). We have previously diagnosed IAD in four patients with primary hypothyroidism and negative TPO antibodies. The aim of this study was to determine the prevalence of undiagnosed IAD in patients with ATD.
Methods
We studied 45 patients with ATD on stable L-thyroxine replacement (dose range 50–350 μg) and 17 healthy subjects (Group A). Fourteen of 45 ATD patients were negative for TPO antibodies; self-reported well-being was impaired in 31 (Group B) and normal in 14 (Group C). All patients underwent adrenal function assessment by a low dose 1 μg short synacthen test; a peak <18 μg/dl was considered as evidence of impaired corticotrophic function and followed up by further investigations. In addition, all patients completed the SF-36 questionnaire.
Results
Peak serum cortisol did not differ significantly between groups (group A: 22.8 ± 3.2 μg/dl, group B: 25.2 ± 4.8 μg/dl, group C 23.0 ± 3.9 μg/dl). Subjective health status according to SF-36 correlated well with self-reported well-being (Group B versus C). The analysis of individual SST responses revealed that one patient with a peak cortisol of 14.5 μg/dl had failed the short synacthen test; she was TPO positive and had complained about impaired well-being. Further work-up with a 250 μg short synacthen test and an ITT confirmed IAD and she was started on hydrocortisone replacement. Intriguingly, in group C baseline ACTH and cortisol levels were significantly lower than in controls (all $P<0.01$) albeit within the normal range, potentially indicating subtle impairment of corticoptrophic function.
Conclusions
Isolated ACTH deficiency may be more common than previously recognized in patients with autoimmune hypothyroidism and impaired well-being, warranting larger studies to define the actual prevalence of IAD in ATD.

P786

From elimination to sustainable control over iodine deficiency in Bulgaria 1997–2006

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Iodine deficiency (ID) and ID disorders (IDD) are a global problem. ID elimination would significantly contribute to achieving at least 6 of the 8 millennium developmental goals.
Aim
To adapt the Neonatal thyroid screening (N?S) based on TSH with a view to its applicability as a permanent indicator in monitoring the effect from the iodine prophylaxis of IDD in Bulgaria.
Tasks
To study: 1) dependence of TSH on age after birth; 2) influence of perinatal application of iodine-containing desinfectants on TSH concentration in newborn children (NB);) TSH – distribution prospectively; 4) commercially available iodized salt according to the new state standard (KIO3 28–55 ppm) and its relation to the percentage of TSH >5 mU/l.
Material and methods
TSH analysis (Delfia) from NTS of 619 898 NB from all over Bulgaria (1997–2006) by specially designed software for registering each screened NB. The TSH concentration of 5 mU/l in $<3\%$ of NB was used as a sign of iodine repletion (WHO proposal).
Statistics: SPSS 10.
Results
Obvious, significant dependency of TSH on age/inversely proportional/and the application of iodine-containing desinfectants/directly proportional. The percentage of NB in Bulgaria with TSH $>$ the suspicious for congenital hypothyroidism (cutoff 15 mU/l) rapidly decreased (1.8–0.09%; $P<0.0001$). The relative share of NB on L-thyroxine treatment increased from 25 to 70%. The significant reduction of NB with TSH >5 mU/l and levels $<3\%$ (first time in 2006) was accompanied by previous stable, sustainable increase of iodized food grade salt above 90%.
Conclusion
Universal salt iodization is an effective strategy for ID elimination. Bulgaria has reached the first stage of sustainable control over ID. NTS, after thorough ?daptation, might be a useful instrument in monitoring the effect of programmes for securing optimal iodine supplementation at population level among the most sensitive to ID individuals represented by the NB.

P787

Should we look for metabolic syndrome (MSy) in subclinical hypothyroidism?

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Subclinical hypothyroidism is a biochemical diagnosis defined with elevated levels of TSH and normal levels of thyroid hormones. The most common abnormality linked to this condition is hypercholesterolemia. The aim of this study was to search for MSy among the persons with subclinical hypothyroidism which was already proven to have hypercholesterolemia when compared to healthy control. MSy was identified in those with elevated triglycerides (Tgc), reduced HDL and elevated fasting glucose. We compared each of these parameters as well as the presence of MSy in patients with subclinical hypothyroidism to age and BMI matched healthy control. We evaluated 60 persons in two groups: 1st group: 38 patients (35 women and 3 men) with subclinical hypothyroidism, mean age 48.63 ± 9.88 years, mean BMI 26.19 ± 6.17 kg/m^2, with laboratory proven subclinical hypothyroidism. Second group: 22 healthy euthyroid controls, mean age 49.14 ± 10.32 years, mean BMI 26.67 ± 4.57 kg/m^2. None of them had a history of coronary heart disease, signs of liver and/or renal dysfunction or were previously treated with lipid lowering drugs. Statistical analysis was performed with *t*-test and Mann–Whitney *U*-test. In the 1st group, mean Tgc level was 2.22 ± 1.74 mmol/l, mean HDL level was 1.23 ± 0.45 mmol/l and mean fasting glucose level was 5.21 ± 0.92 mmol/l. In the 2nd group, mean Tgc level was 1.36 ± 0.42 mmol/l, mean HDL was 1.39 ± 0.35 mmol/l and mean fasting glucose was 4.63 ± 0.41 mmol/l. There was a high statistical difference between the level of tgc between the groups ($P<0.01$). For the level of fasting glucose, there was a statistical difference ($P<0.05$). For the levels of HDL and the overall presence of MSy between the groups there was no statistical difference ($P>0.05$). Eventhough there was no difference in HDL levels and no difference in the overall presence of MSy between the groups we think that along with hypelipidemia MSy is something to think about in subclinical hypothyroidism.

P788

Hurthle cells tumors of the thyroid: personal experience at the Regina Elena Cancer Institute, Rome (Italy)

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Hurthle cell carcinoma (HCC) represents about 5% of differentiated thyroid carcinomas. When Hurtle cell represents more than 75% of cells population, the lesion can be considered a Hurthle cell tumor (HCT); it can be classify as malignant when capsular or vascular invasion is reported or if there is a perithyroid infiltration or distant metastases occur. Aim of this study was to present our own experience on the clinical and pathological features of patients (pts) affected by HTC that can predict disease progression and death. Age, disease stage, tumor size, extra glandular invasion, lymph-nodes disease, distant metastases, extensive surgery, radioiodine therapy and external beam radiation therapy as factors potentially associated with decreased survival were evaluated for all patients. We have identified 28 pts affected by Hurthle cell tumor, 9 with HCA and 19 with HCC (22 F, 6 M) mean age of HCT pts. was 49.7 years (30–72 years) versus 49.3 years (15–72 years) of HCC. In all pts, a total thyroidectomy was performed. At hystology were found 9 adenomas, 5 'minimally invasive' and 14 invasive carcinomas. Average size of primary tumor was: 28.8 mm medium diameter in HCT versus 25.8 mm in HCC. None patients had lymph-nodes metastases. HCC patients TNM staging showed 9 patients stage I, 5 stage II, 4 stage III and 1 stage IVa (UICC 2002). All invasive carcinomas underwent ^{131}I therapy (91–150 mCi). One HCC patient received external beam radiotherapy. The average follow-up period was 62 months (range 6–324). In none of our cases, with adenoma a relapse was observed. Only one HCC patient showed distant lung metastases at 60 months of follow up. In conclusion, HCC was not found to have an aggressive behaviour. None of HCT showed a relapse

P789

Radioiodine therapy in patients with toxic nodular goitre

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The aim of our study was to assess the effectiveness of radioiodine therapy (RIT) on the achievement of euthyroidism and reduction of thyroid volume, in patients with toxic nodular goitre (TNG).
Material and methods
During the last 7 years, we treated 3800 patients with TNG, aged 30-70 years; 82% female and 18% male; 2200 patients with multinodular goitre (MNG) and 1600 with autonomous toxic nodule (ATN); thyroid volume ranged between 16 and 130 ml (30% with thyroid volume >60 ml).
Qualification of these patients were based on clinical features, characteristic appearance on thyroid scans and ultrasound. Malignant changes were excluded in all nodules by fine needle aspiration biopsy. All the patients had serum TSH levels below 0.1 mU/l and effective half-life more than 3 days at the time of treatment. The activity dose was calculated by Marinelli's formula and ranged between 200 and 800 MBq. The absorbed dose (Gy) ranged between 150 and 260 for MNG, and 200-300 for ATN. Follow-up control was done every 6 weeks. Thyroid ultrasound, and thyroid scan were done before and after12 months of RIT to assess RAIU, volume of thyroid gland and nodules. Repeated RIT was given after 6 months of the first dose if needed.
Results
After 4 years of follow-up, the success of treatment was: 97% of patients with ATN and 92% of patients with MNG achieved euthyroidism. Three percent of patient with ATN and 8% of patient with MNG develop hypothyroidism. Thirty-four patients with toxic MNG and 5 patients with ATN received more than one dose of RIT. Thyroid volume reduced to 52% in MNG and 47% in ATN.
Conclusions
The achievement of euthyroidism and the remission of the symptoms and signs of clinical hyperthyroidism, were due to well preparation of the patients; accurate measurement of administered activity, relatively high effective half-life, and well-organised follow-up.

P790

A case of thyroid abscess due to piriform sinus fistula complicated by hyperthyroidism

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Acute suppurative thyroiditis is a rare disease because thyroid gland is highly resistant to infection. The most common predisposing factor is the presence of a piriform fistula. We report an unusual case of hyperthyroidism caused by AST.

A 27-year-old woman was admitted to our hospital because she presented a painful swelling in the left thyroid gland with fevers. Laboratory test showed a white leukocyte count of 14 000/ul, a CRP level of 251 mg/l, a thyrotropin level of 0.1 mUI/l, a T4 level of 41 pmol/l. A neck computed tomography scan revealed an abscess in the thyroid gland and the presence of a piriform sinus fistula. After appropriate antibiotic therapy and propanolol administration, the patient underwent fistulectomy and resection of the left upper lobe of her thyroid gland. The thyroid function became normal approximately one month after.

So the clinical feature of thyroid abscess includes hyperthyroidism. It can be evocated before AST because some cases of thyrotoxicosis have been described and can complicated surgery.

P791

Cognitive dysfunctions during chronic thyrotropin-suppressive therapy with levothyroxine in patients with differentiated thyroid carcinoma

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Background

TSH-suppressive therapy is widely used in treatment of thyroid differentiated carcinoma. A common consequence of therapy is subclinical hyperthyroidism which may cause dysfunction of cardiovascular system, metabolism and reduction of bone mass[1]. Thyroid hormones are also involved in regulation of brain function[2]. Therefore, it is not surprising that thyroid dysfunctions are associated with frequent comorbid cognitive dysfunctions and depression.

The aim of our study was to assess the cognitive functions in patients treated with suppressive doses of levothyroxine due to thyroid papillary carcinoma.

Method

Twenty-eight patients with subclinical hyperthyroidism in the course of substitutive treatment with levothyroxine due to total thyroidectomy and 131I therapy were involved in the study. The control group consisted of 17 healthy, euthyroid subjects. A battery of neuropsychological tests was administered to assess: 1. Working memory and executive functions (the Wisconsin Card Sorting Test – WCST, The Controlled Oral Word Association Test-FAS), 2. psychomotor speed (the Trial Making Test – TMT) 3. attention (the Stroop test) and 4. Short term memory (the Digit Span test). Psychometric evaluation was made using 17 items the Hamilton Depression Rating Scale and Beck Depression Inventory.

Results

Patients compared to control group performed poorer in WCST. They made significantly more perseverative errors. Patients were found to perform less well than controls in FAS and in TMT-B. The mean score of HDRS and BI (3.4 and 6.6 respectively) suggest that patients were not depressed during examination.

Conclusion

Our results suggest that suppressive treatment with levothyroxine may affect executive functions, working memory, psychomotor speed.

References

1. Biondi B, Palmieri EA, Klain M, Schlumberger M, Filetti S & Lombardi G. Subclinical hyperthyroidism: clinical features and treatment options. *Eur J Endocrinol* 2005 **152** 1–9.
2. Smith JW, Evans T, Costall B & Smythe JW. Thyroid hormones, brain function and cognition: a brief review. *Neurosci Biobehav Rev* 2002 **26** 45–60.

P792

Serum ghrelin levels in thyroid dysfunction and its change with treatment

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Aim

In this study, we aimed to detect the serum Ghr levels in hypo and hyperthyroid patients at the time of diagnosis and after treatment in comparison with an age-, and sex-matched control group.

Materials and methods

Thirty-two hypothyroid, 19 hyperthyroid and 30 control subjects were included in the study. Basal levels of serum free T3, free T4, TSH, anti-thyroid peroxidase (anti-TPO), anti Tg antibodies were measured by commercially available kits in all patients and control group hypothyroid patients were treated with levothyroxine, patients with Graves' disease were treated with methimazole and propranolol, patients with thyroiditis were managed with propranolol only. Patients were followed for three months. Serum free thyroid hormone levels and TSH were measured at the first and the third months of the treatment. Serum levels of Ghr were measured at the time of diagnosis, at first and the third months in patients with thyroid dysfuntion. Serum IGF-1 levels were determined at the time of diagnosis and following 3 months of treatment.

Results

Serum Ghr levels in hypothyroid patients were lower than the control group at the time of diagnosis and decreased more following treatment. Serum Ghr levels in hyperthyroid patients were lower than the control group at the time of diagnosis, but did not normalize after euthyroidism was achieved. No statistically significant correlation was detected between Ghr and patient age, BMI, free T3 or TSH. There was not a significant correlation between Ghr and pretreatment free T4 levels in both patient groups and the control group, but Ghr was negatively correlated with posttreatment free T4 at the first and the third month in both patient groups.

Conclusion

Ghr is affected in thyroid dysfunction which may be directly due to the effects of free thyroid hormones or due to the secondary weight changes or both. But further studies are warranted to determine the net effect of thyroid hormones on Ghr.

P793

Hyperthyroidism associated to hyperthyrotropinemia: differential diagnosis between resistance to thyroid hormone and TSH secreting pituitary adenoma

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Hyperthyroidism associated to hyperthyrotropinemia is a rare condition which can be due to two different causes: resistance to thyroid hormones and TSH secreting adenoma. The differential diagnosis is not easy.

We have observed 8 patients with hyperthyroidism associated to inappropriately elevated TSH. All patients had similar basal levels of thyroid hormones and 7 out of 8 had a multinodular goiter.

Four out of 8 patients showed lack of TSH response to TRH stimulation. In all these cases MRI showed a pituitary adenoma. Two of them also showed lack of GH suppressibility after oral glucose load, suggestive of mixed adenoma secreting GH and TSH. Three out of 4 patients underwent successfull transsphenoidal surgery while one patient, affected by a mixed adenoma, was successfully treated by octreotide.

In the other 4 patients, TSH response to TRH stimulation was present, but 1 of them showed a pituitary adenoma. The thyrotoxicosis persisted after adenomectomy despite the decrease of TSH levels. Thyroidectomy was performed with histological diagnosis of Graves' disease.

Three out of 4 patients were diagnosed as resistance to thyroid hormones. Two of them underwent thyroidectomy because of goiter. A papillary carcinoma was

diagnosed in 1. This patient, because of the impossibility of obtaining a proper TSH suppression, represents a unique model of thyroid cancer follow-up under TSH constantly elevated.
These cases demonstrate that differential diagnosis of hyperthyroidism associated to hyperthyrotropinemia is hard to be done. It is necessary to evaluate clinical features, laboratory data and imaging. An adequate response to TRH in presence of mild clinical features is suggestive of resistance to thyroid hormones, while in case of overt hyperthyroidism other etiologies should be excluded. MRI scan is essential for diagnosis of TSH secreting adenoma.

P794

Decreased fibrinolytic activity due to levothyroxine suppression therapy for benign thyroid nodules

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Thyroid dysfunction is associated with altered levels of several coagulation factors. The aim of this study is to investigate the effect of levothyroxine suppression therapy for benign thyroid nodules on coagulation system.
Thirty-six patients with benign thyroid nodules, who were applicant for levothyroxine suppression therapy, were included in the study. Thirty patients completed 1 year follow-up period. Levothyroxine was given to maintain TSH level between 0.1 and 0.35 mIU/l. Samples were collected before and after therapy.
No significant decrease in diameters and volumes of 128 thyroid nodules were observed after one year levothyroxine suppression ($P>0.05$). No alteration was found in plasma tPA ve tfPI levels after therapy ($P>0.05$). However, plasma fibrinogen ($P<0.001$), d-dimer ($P<0.001$), vWF ($P=0.025$), and PAI-1 antigen levels ($P=0.032$) increased after levothyroxine suppression. On the other hand, plasma TAFI antigen levels decreased significantly ($P=0.005$).
Our results suggest a decrease in fibrinolytic activity after levothyroxine suppression therapy for benign thyroid nodules. The decrease in TAFI antigen levels may be due to activation of TAFI pathway.

P795

Graves' disease and myasthenia gravis: who comes first?

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Graves' disease (GD) and myasthenia gravis (MG) may be associated and influence one another clinical expression. We report the cases of 3 female patients who presented this particular association. First patient (RL, 55 years), was diagnosed with MG and, 5 years after, developed GD, remitted after 2 years of anti-thyroid drugs (ATD). She is still euthyroid after more than 10 years, and MG is compensated with moderate doses of anticholinesterase drugs (ACD). Thoracic CT showed stable thymus hyperplasia. Second patient (AI, 42 years) have been diagnosed with both diseases practically in the same time: suspicion of MG, positive electromyography, clinical symptoms suggesting GD with biological confirmation. She had normal thoracic CT. She had a more difficult evolution, with cardiac failure, rapid installation of hypothyroidism on medium doses of ATD and persistence of important neuro-muscular symptoms, which made necessary near-total thyroidectomy. Post-surgical she presented higher LT4 necessary (200 mcg/d) but MG was spectacularly improved. Third patient (MM, 56 years) had a first episode of GD and, 5 years later, relapse of GD and onset of MG. Near-total thyroidectomy was performed, with post-surgical hypothyroidism compensate with 75 mcg LT4. However, MG symptoms persisted, and needed higher doses of ACD. Thoracic CT showed thymus tumour which was resected. The histology showed thymolipoma and the necessary ACD doses remained unchanged. The association of GD and MG is challenging because the evolution of one disease may be influenced by the other. Two of our patients had an improvement of the MG evolution after remission of GD but neither thyroidectomy nor thymectomy could diminish the ACD needs for the third. The diagnosis of MG had preceded, followed or been contemporary to the GD diagnosis. No matter who comes first, one should have in mind this association and adjust the management to the specific evolution.

P796

Iodine deficiency disorders in eastern Ukraine

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Background
Ukraine does not yet have a national program for control of iodine deficiency and there are no recent data on the severity of the iodine deficiency disorders (IDD) in the country.
Objective
The aim of the present study was to assess current IDD status in eastern Ukraine.
Design
We conducted school-based 30-cluster survey in children 6-11-year-old in urban and rural areas. We measured urinary iodine concentration (UI) and iodine content of salt by rapid test kits. Goiter was graded by palpation and thyroid volume determined by ultrasound. We measured hemoglobin (all children), serum ferritin, serum transferrin receptor, thyrotropin, thyroxine in children with goiter and anemia.
Results
Children were sampled at 20 urban and 10 rural primary schools. In the 20 urban schools, the median UI was 89 μg/l, 36% of household salt samples were iodized (adequate levels), the goiter prevalence was 32%. In the rural schools, the median UI was 76 μg/l, the goiter prevalence was 18 and 12% household salt samples were adequately iodized. Among children in the rural schools anemia prevalence was 12%, in urban schools – 36%.
Conclusions
In eastern Ukraine, school children in all areas are mildly iodine deficient. But the prevalence of IDD is higher in children in urban school. Coexisting deficiencies of iodine and iron and severe environmental conditions at the industrial cities can impair thyroid function.

P797

The effect of radioiodine therapy in patient with subclinical hyperthyroidism

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The aim of our study was to assess the influence of radioiodine (^{131}I) therapy on the achievement of euthyroidism, prevention of adverse effects on the cardiovascular and skeletal systems and prevent evolvement to overt hyperthyroidism.
Material and methods
We treated 630 patients, aged 30–70 years; 85% of them were female and 15% male; 220 patient with multinodular goitre (MNG), and 310 patient with autonomous nodule (ATN). Some of the patients were treated with antithyroid drugs for 6 to 24 months before I^{131} therapy (110 patient). Malignant changes were excluded in all nodules by fine needle aspiration biopsy. All the patients had serum TSH levels <0.1 mU/l and effective T-half was more than 3 days at the time of treatment. The activity dose was calculated by the use of Marinelli's formula and ranged between 200 and 600 MBq. The absorbed dose (Gy) ranged between 150 and 300, and was proportional to thyroid volume. Follow up control was done every 6 weeks.
Results
Euthyroidism achieved in 99% of patient with ATN and 95% of MNG; 1% of patient with ATN and 5% of patient with MNG develop hypothyroidism. In all of the patients, the symptoms and signs of subclinical hyperthyroidism disappeared (palpitation, tachycardia, atrial fibrillation, exercise tolerance improved, the blood pressure normalised and the quality of life improved). One percent of the patients received 2nd dose of radioiodine.
Conclusions
Our result is good and is in the range of the existing literature. The achievement of euthyroidism and the remission of the symptoms and signs of subclinical hyperthyroidism, were due to good diagnosis, well preparation of the patients; accurate measurement of administered activity, effective half-life, and well-organised follow up. We recommend early treatment of subclinical hyperthyroidism, and long period of follow up visits in our department (up to 10 years) to evaluate the long term effect of RIT.

P798

Abstract unavailable

P799

Thyroid hormone abnormalities in chronic renal failure

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In 162 patients with end-stage renal disease (ESRD), 112 on maintenance haemodialysis (HD) and 50 on continuous ambulatory peritoneal dialysis (CAPD), thyroid status was evaluated: thyrotropin (TSH, IRMA, normal range 0.15–5 mU/l), total thyroxine (TT4, RIA, 70–140 nmol/l), free thyroxine (FT4, RIA, 11–25 pmol/l), total triiodothyronine (TT3, RIA, 1–3 nmol/l), free thyroxine (FT3, RIA, 2.5–5.8 pmol/l); in 113 patients (63 + 50) also reverse T3 (RT3, RIA, 0.14–0.54 nmol/l) was assayed. None of the patients had previously been treated for or suspected of a thyroid dysfunction.
Among HD patients, 11.6% had higher TSH, 38.4% lower TT4, 56.3% lower FT4, 21.4% lower TT3, 19.6% lower FT3, and 17.5% higher RT3 than the respective normal range. In CAPD patients, the corresponding values were 20, 8, 62, 16, 10, and 4%. There was a significant difference between HD and CAPD groups in TSH (medians 2.07 and 3.12, respectively, $P<0.001$, Mann–Whitney), in TT4 (77.5 and 107.3, $P<0.001$), in FT3 (3.17 and 3.82, $P=0.019$), and in RT3 (0.34 and 0.28, $P=0.004$). Both in HD and CAPD patients there were highly significant correlations (all $P<0.001$) between TT4 and FT4 (Spearman rho 0.658 and 0.674, in HD and CAPD respectively), and between TT4 and TT3 (0.355 and 0.526). Less expectedly, there were also strong correlations between TT4 and RT3 (0.605 and 0.695), and FT4 and RT3 (0.700 and 0.674).
Our data confirm the frequent deviations from normal values in ESRD patients, reflecting the complex alterations in thyroid hormone metabolism as well as assay-dependent variations. The pattern of non-thyroidal illness was more expressed in HD than in CAPD patients, including an increase in RT3, rarely observed in renal disorders. The close correlation between T4 and RT3 may theoretically reflect variations in deiodinase I activity among patients with kidney failure.

P800

Insulin resistance and oxidative stress induce advanced glycation end products formation in patients with clinical and subclinical hypothyroidism

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Introduction
Advanced glycation end products (AGEs) formation is accelerated in various pathological conditions characterized by insulin resistance (IR) and/or increased oxidative stress (OS). Hypothyroidism – overt (OH) or subclinical (SUH) – is associated with a variety of metabolic disorders leading to IR and increased OS.
Aim
To estimate the $^{\varepsilon}N$-carboxymethyl-lysine (CML) levels, in subjects with OH and SUH and seek for possible correlations with various metabolic parameters including IR and OS.
Subjects and methods
We studied patients with OH ($n=15$), with surgically induced OH (SOH) ($n=15$) and SUH ($n=15$), mean age 43 ± 10 years (TSH: 40 ± 10, 79 ± 21, 7.7 ± 3.4 μIU/ml, respectively). Fifty healthy subjects (C) matched for age and BMI, were also studied. Thyroid hormone levels, biochemical parameters, creatinine clearance and microalbuminuria were measured by standard laboratory techniques. CML and 8-isoprostanes levels were determined by ELISA. Dietary AGE intake (dAGE) was estimated by 3-day dietary records and specific questionnaires. The IR was estimated by HOMA index ($G_{fasting}\chi I_{fasting}$)/22.5).
Results
HOMA was higher in OH, SOH and SUH groups compared to C (2.4 ± 1, 2 ± 1, 2.2 ± 0.7, 1.4 ± 0.6, respectively, $P<0.05$). Levels of 8-isoprostanes were higher only in OH and SUH compared to C (230 ± 44, 214 ± 86, 133 ± 69 pg/ml, respectively, $P<0.05$). Higher CML levels were observed in OH, SOH and SUH groups compared to C (15 ± 5, 13 ± 4, 11 ± 4 U/ml, respectively, $P<0.005$) with positive correlation to HOMA index and 8-isoprostanes levels in all groups except the SOH ($r=0.6$, $P<0.03$, respectively). All the subjects had normal renal function and did not exhibit statistically different dAGE values.
Conclusion
Increased OS and IR possibly induce increased AGE formation in OH and SUH, which might contribute to the enhanced risk of cardiovascular disease. However, other mechanisms such as disrupted function of the AGE receptors cannot be excluded and warrant further investigation.

P801

TSH levels and thyroid nodules correlation in autoimmune thyroiditis

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The role of TSH in the pathogenesis of thyroid nodule (TN) is controversial.
We have evaluated the prevalence of TN in a group of 800 subjects with and without autoimmune thyroiditis (AT). The prevalence of TN, the number and volume of nodules was evaluated in relation with TSH and antithyroid antibodies titles.
The subjects were of both sex, aged between 20 and 80 years and were recruited in a random manner, in the context of an epidemiological study (FATA); nobody were under farmacologic treatment.
All the subjects were submitted to an echographic study of the neck region and to determination of TSH and antithyroid antibodies (TAb).
We found in the total population a prevalence of TN of 26.8%; TN prevalence was significantly higher ($P<0.01$) in the subjects with TSH levels less to 4.5 mUI/ml (29.1%) than those with above 4.5 mUI/ml (25.9%); the difference in the prevalence of TN between thyroid antibodies (Ab+) respect to (Ab−) subjects, is not significative.
A negative correlation was found between TSH levels and number of thyroid nodules ($r=-0.245$ $P<0.001$); while there was no correlation with the levels of antithyroid antibodies.
Related to volumes of TN the subjects with TSH levels above 4.5 mUI/ml showed a mean TNV of 17.7 cc., significantly reduced ($P=0.000$) respect to the subjects with TSH below 4.5 mUI/ml (mean TNV of 20.95 cc). A statistically difference ($P=0.05$) was also observed in the mean TNV between subjects Ab+ (20.01 cc) related to those Ab− (20.96 cc).
Results show TSH seems to play no role in the TN growth, because higher levels are not associated to bigger TN, while TAb presence determines a lesser TN growth.

P802

Serum thyrotropin concentration as a biochemical predictor of thyroid malignancy in patients presenting with thyroid nodules

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Background
Fine-needle aspiration biopsy is the 'gold standard' in the preoperative management of thyroid nodules.
Aim
The aim of this study was to investigate whether serum TSH is a predictor of thyroid malignancy in patients presenting with thyroid nodules.
Subjects and methods
About 565 patients without overt thyroid dysfunction, who presented with palpable thyroid nodule(s) between 1988 and 2004 and underwent at least one FNAB, were retrospectively evaluated.
Results
The final diagnostic outcome was established after surgery ($n=122$) or after a minimum of one-year clinical follow-up period. Higher rates of malignancy were observed in patients with serum TSH in the upper tertile of the normal range ($P=0.026$). Binary logistic regression analysis revealed significantly increased adjusted odds ratios for the diagnosis of malignancy in patients with serum TSH 1.5–4.0 mIU/l compared to those with either TSH 0.4–0.8 mIU/l ($P=0.005$) or TSH 0.9–1.4 mIU/l ($P=0.007$).
Conclusions
The risk of malignancy in thyroid nodules increases in parallel with TSH concentrations within the normal range. TSH concentration at presentation is an independent predictor of thyroid malignancy.

P803

Thyroid peroxidase antibodies and levels of thyroid stimulating hormone as predictors of development of hypothyroidism in euthyroid subjects

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Introduction
Presence of thyroid peroxidase antibodies (TPOAbs) may be a marker of future thyroid failure. However, prospective studies in a euthyroid population have not been performed.
Aim of the study
To prospectively investigate the relationship between presence and levels of TPOAbs and incident hypothyroidism in euthyroid subjects in the general population.
Study population
The database used for this study consisted of a random sample of 2703 participants of the PREVEND (prevention of renal and vascular end stage disease) study, inhabitants aged 28–75 years of the city of Groningen, The Netherlands. We excluded subjects with a TSH level outside the laboratory's reference range (0.35–4.94 mIU/l; $n=115$) at baseline and subjects taking thyroid medication ($n=37$) and/or medications that may affect thyroid function ($n=56$). Incident hypothyroidism was defined as initiation of L-thyroxine therapy in the absence of thyreostatic medication by the participants during follow-up.
Results
Prevalence of positive TPOAbs at baseline was 8.5%, with levels between 12 and 3767 IU/ml. Prevalence significantly increased with increasing TSH concentrations ($P<0.001$). Median age at baseline was 46 years. Median follow up was 6.7 years. Incidence of hypothyroidism was 0.5% (3.0% in TPOAbs positive versus 0.2% in TPOAbs negative subjects; $P<0.001$). Sex (HR 8.6 (1.10–67.17), $P=0.04$), TSH level (HR 3.4 (2.07–5.60), $P<0.001$) and TPOAbs level (log transformed; HR 3.93 (2.26–6.82), $P<0.001$) were significant predictors of incident hypothyroidism in univariate analysis. Age was not related. When tested multivariately, the product term of TSH level and TPOAbs level was significantly related to incident hypothyroid cases ($P=0.01$), reflecting highest risk in subjects with both high levels of TPOAbs and high levels of TSH within the reference range.
Conclusion
We have demonstrated that TPOAbs level and TSH level are interdependent predictors for future hypothyroidism, even when TSH is still within the laboratory's reference range.

P804

The effect of radioiodine therapy in patient with non-toxic goitre after pre-treatment with a single dose of recombinant human thyroid stimulating hormone (rhTSH)

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The aim of our study was to assess the effectiveness of radioiodine therapy (RIT) on the reduction of thyroid volume after pre-treatment adjunct of rhTSH in patients with non-toxic goitre with low RAIU.
Material and methods
We treated 36 patients; (28 female, 8 male) aged 35–77 years. Initial 24 h RAIU was ranged between 5 and 17%, and thyroid volume ranged between 42 and 128 ml. Twelve patients had compressive symptoms. Malignant changes were excluded in all nodules by FNAB. All the patients received a single dose of 0.05 mg rhTSH given intramuscular. About 24 h later diagnostic dose of ^{131}I was administered and RAIU after 24, 48 and 72 h was estimated. Therapeutic dose of ^{131}I was given on the third day of rhTSH administration. Serum TSH, fT4 and fT3 were determined, 24 h, 72 h after rhTSH administration and on the 3rd day after RIT. The activity dose calculated by Marinelli's formula and ranged between 400 and 800 MBq. The absorbed dose ranged between 160 and 300 Gy. Follow up control was done every 6 weeks. Thyroid ultrasound, and thyroid scan were done again after12 months of RIT.
Results
A significant 4-fold increase in 24 h RAIU from 12.2 to 54% was observed. The significant increase in serum TSH from 1.4 ± 0.5 to a peak level 12.21 ± 4.62 was seen after 24h . After 12 months 91% of patient were in euthyroidism, 9% (3 patients) develop hypothyroidism. Thyroid volume reduced to about 45% average. In all of the patients the compressive symptoms relieved and exercise tolerance improved.
Conclusions
Pre-treatment with rhTSH allows the therapeutic dose of ^{131}I to be reduced by 50–58% without compromising the result of thyroid volume reduction. This mode of therapy can be recommended, especially when RAIU is low and the dose of radioiodine to be administered is high.

P805

High prevalence of pathologic TSH values in patients admitted to a clinic of general internal medicine

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Currently there are only rare data on the prevalence of thyroid dysfunction in hospitalized patients. Therefore, we prospectively measured TSH values in patients admitted to a clinic of general internal medicine in an area of moderate iodine deficiency.
In 1011 patients (mean age 66.2 years; median 72 years) TSH was consecutively evaluated.
The mean TSH was 1.74 uU/ml (range 0.00–93.3); median 1.1 uU/ml.
In 732 patients (74.4%) TSH was within the normal range (0.35–2.8 uU/ml) 120 patients (11.9%) had TSH levels above 2.8 uU/ml.
About 139 patients (13.7%) had TSH levels below 0.35 uU/ml. Among those 139 patients 51 patients had TSH values below 0.1 uU/ml.
There was a negative correlation of age and TSH ($P<0.05$).
We found pathologic TSH values in 25% of patients admitted to a clinic of general internal medicine. Whether these alterations are related to true thyroid dysfunction or are influenced by the underlying illness needs further evaluation.

P806

A rare clinical picture: thyroid cystic nodule as a localization of Aspergillus pulmonary infection (pulmonary-thyroid form)

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Aspergillus infection (A)is often recognized in severely immunocompromised patients.
Although involvement of the thyroid gland is reported for 9–15% of patients with disseminated disease, a localized clinical picture it's not reported.
Here, we present a case of *Aspergillus* in a LES young patient (15-year-old girl), who underwent to a strong immunosuppressive regimen (high doses of corticosteroids, endoxan and plasmaferesis). After about one month she developed pulmonary A. Chest CAT revealed signs of A infection (interstitial and cavernous form). Routine respiratory cultures repeatedly grew *A. flavus*.
The galactomannan enzyme-linked immunosorbent assay, one of the most sensitive test available for aspergillosis diagnosis, had a positive result.
Laboratory tests performed before A development, in order to test autoimmune associated diseases, showed TSH mild elevated (8.5 mUi/ml), normal FT3 (2.38 pg/ml) and FT4 (1.07 ng/dl) AbTg (3.7 UI/ml) AbTPO (33 UI/ml), suggesting a form of autoimmune thyroiditis.
She was treated with L-T4. When A has arisen, at physical examination of the neck a small nodule was appreciated.
An anecoic imagine was shown by ultrasound, no colour signs at ecocolor-doppler A FNA was made and septate hyphae, consistent with *Aspergillus* species, were identified in Periodic acid-Schiff stain cytology.
The patient was put in antifungeal therapy and the thyroid nodule was monitored. The A. pulmonary picture remitted, while the thyroid cyst is still evident at ecography, presenting the same size and ecostructure, and at FNA positive Periodic acid-Schiff stain cytology for hyphae.
The involvement of thyroid by A is frequent, mostly in the context of a A diffusive form. This is characterized by a destructive form of thyroiditis.
A localized form as a thyroid cystic nodule, like we described here, associated to pulmonary A (pulmonary-thyroid form) is unusual. It's interesting it seems to show a some resistance to antifungeal therapy.

P807

Clinical variables associated with recurrence of hyperthyroidism in Graves' disease

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Introduction
In Graves' hyperthyroidism with long-term antithyroid drugs (ATD) treatment, remission is often unpredictable. However variables at diagnosis or at the end of the ATD regimen are associated with a highest likelihood of recurrence and could be used to decide an ablative treatment.
Objective
To identify variables before and at the end of a long-term treatment with ATD (>6 months) associated with higher rates of relapse during a follow-up of at less 1 year after discontinuing ATD.
Subjects
About 250 subjects with a first episode of Graves' disease between January 1999 and December 2004 seen in the same institution.
Results
At baseline mean age was 41 ± 14 years, 21% were male and 33% smokers, 49% used iodinated salt and 40% were classified as having a goiter $>$ grade 2. Mean TSI decreased from 11 ± 18 to 2.5 ± 4 ($P<0.05$) at the end of ATD regimen. About 29% were excluded of a further analysis because of an ablative treatment as soon as ATD were stopped. Relapse ocurred in 60% of the resting 177 subjects submitted to a long-term treatment (mean 18 ± 8 months) during the follow-up period (mean: 3.1 ± 1.7 years). Of analyzed variables at diagnosis,only male gender, smoking history and iodinated salt use were significantly associated with failure of medical treatment. In a logistic regression analysis only male gender and smoking cigarettes were independent predictive factors for recurrence. TSI 3 times above the normal value at the end of the treatment yield a 98% positive predictive value for relapse.
Conclusion
Recognition of variables at diagnosis of Graves' disease or at the end of long-term ATD course could be used to select pacients for surgery or radioiodine because of lower remission rate with medical treatment.

P808

Is pre-ablative thyroglobulin useful as a predictor of cure in differentiated thyroid carcinoma?

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Surgery followed by ^{131}I ablative therapy (IAT) is the usual treatment for patients with differentiated thyroid carcinoma (DTC). Thyroglobulin levels obtained 9–12 months after IAT under TSH stimulation (Tg2) is a very reliable marker for the presence of thyroid tissue. The value of stimulated thyroglobulin previous to ablation (Tg1) is more controversial. The aim of this study was to examine the relationship between Tg1 and Tg2.
Material and methods
Seventy-five patients treated for a DTC between 2001 and 2006 were studied. All patients had undergone total o near-total thyroidectomy followed by IAT after thyroid hormone withdrawl or stimulation with Thyrogen. Serum TSH, Tg and Tg antibodies (Tg Ab) were measured postoperatively just before IAT or three days after the second dose of Thyrogen and 9-12 months after ablation with stimulation of TSH. Serum Tg was measured by inmunoradiometric assay with functional sensitivity of 0.5 ng/ml.
Results
Twenty-four patients were excluded: 1 with evidence of macroscopic disease, 5 with TgAb positive, 8 patients in which TgAb were not measured and 10 who had not Tg2 done. The remaining 51 were divided in three groups as follows: Group 1 ($n=11$) Tg1 between 0 and 1 ng/ml: all of them had Tg2 <1 ng/ml; Group 2 ($n=21$) Tg1 between 1 and 5 ng/ml: 2 presented Tg2 >1 ng/ml; Group 3 ($n=19$) Tg1 >5 ng/ml: 6 with Tg2 >1 ng/ml. ^{131}I uptake outside the thyroid bed was demonstrated in 3 patients in group 1, 5 in group 2 and 10 in group 3.
Discussion
The prognostic value of the Tg levels measured just before IAT is often debated because of the presence of thyroid remnants that contribute to the Tg synthesis. In our series, patients with Tg levels <2 ng/ml before IAT did not show Tg >1 ng/ml 9–12 months post ablation.

P809

Simultaneous papillary (PTC) and medullary (MTC) thyroid cancer: more than a coincidence? Report of two cases

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The simultaneous occurrence of MTC and PTC in the same thyroid can be observed as a mixed tumour folliculo-medullar (OMS 1988) or as a collision tumour separated by normal thyroid parenchyma, which has only been described in less than thirty patients. We report two cases.
Case 1
A 72 years old woman who underwent total thyroidectomy for a multinodular goiter. Histology showed a 2.5 cm CPT in the left lobe and a 4.5 cm CMT in the right lobe. She received 100 mCi ^{131}I. After 2 years serum thyroglobulin (Tg) levels remain undetectable, calcitonin $=11$ (normal <12 pg/ml) and has a negative neck ultrasound. The RET proto-oncogen study did not show any mutation.
Case 2
A 43 years old man harbouring a RET mutation at codon 611 who underwent total thyroidectomy and central neck dissection. Histology showed a 0.3 cm CPT in the left lobe and a multifocal bilateral CMT (0.3 cm and <0.1 cm). Four out of five cervical lymph nodes contained MTC. He received 100 mCi ^{131}I. Tg pre-ablation levels were 0.9 ng/ml and he showed cervical lymph nodes ^{131}I uptake. After 4 months serum calcitonin levels remain below 4 pg/ml.
Discussion
We report two cases of collision tumour with special features. As far as case 1 is concerned the size of the CPT is the biggest one to our knowledge and the outcome is good despite the tumour size and the patient's age. In case 2 the RET mutation at codon 611 has not been previously described since all tumour collision had been associated with mutations at codon 790, 791 and 804. We want

to emphasize the good response to treatment despite the aggressive presentation. The simultaneous occurrence of MTC and PTC may be due to a common genetic drive although a coincidence cannot be ruled out.

P810

Effects of rituximab (RTX) treatment on IL-6 and soluble IL-6 receptor secretion in patients with thyroid-associated ophthalmopathy (TAO)

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We described a significant clinical response to RTX treatment in patients with active TAO. In order to understand the possible mechanisms of action of RTX, we measured serum concentrations of IL-6 and its soluble receptor (sIL-6R). We treated 10 patients with RTX: 8 women, 2 men aged 31-51 yr with Graves' disease. We also treated 14 patients with i.v. steroids (IVGC). All had active TAO. In all patients we studied thyroid function, serum autoantibodies and peripheral blood lymphocytes and by ophthalmologic evaluation the clinical activity score (CAS). Patients were treated with RTX (1000 mg i.v. twice at 2-week interval) or with IVGC (500 mg i.v. for 16 weeks). Serum cytokines were measured by highly sensitive assays (Quantikine, R&D Systems, USA) at baseline and at the time of CD20+ depletion, 30 and 50 weeks from therapy. Basal serum IL-6 concentrations were 30.6 ± 83.6 and 9.4 ± 17.4. in patients treated with RTX and IVGC respectively, and did not change after therapy (ANOVA; P=NS). Serum IL-6 did not change in relation to CD20+ depletion (ANOVA; $P=0.17$). There was no correlation between serum IL-6 and either thyroid autoantibodies or the CAS. Mean basal serum sIL-6R concentrations were 478.2 ± 132.3 and 578.6 ± 172.7 pg/ml in patients treated with RTX and IVGC respectively, and did not change after therapy (Anova). Serum sIL-6R did not change in relation to CD20+ depletion (ANOVA; $P=0.30$). There was no correlation between serum sIL-6R and either thyroid autoantibodies or the CAS (P=NS) We did not observe any significant change of either serum IL-6 or sIL-6R with respect to the modality of treatment (ANOVA). These findings suggest that: a) the RTX effect on TAO does not seem to involve the IL-6/sIL-6R system; b) the therapeutic action of IVGC is also not modifying the secretion of these cytokines; and c) the beneficial effects of RTX may be solely related to the consequences of B cell depletion.

P811

Papillary thyroid microcarcinoma in 188 consecutive thyroidectomies

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Objective
Papillary thyroid microcarcinoma (PTM) is a malignant thyroid tumor with potential multifocality and diameter ≤ 1 cm. This carcinoma has been discovered more frequently like incidentaloma, and the epidemiology is not clearly established. We have analysed epidemiologic and clinical characteristics in La Palma Island.
Patients and methods
We collected all cases of PTM diagnosed in 188 consecutive thyroidectomies performed for whichever cause, admitted from 2000 to 2007. We reported the pre-surgical diagnosis, sex, age at thyroidectomy, thyroid function, thyroid autoimmunity, tumor size, multifocality, tumor extension, and cytology and pathology results. The prevalence and incidence were expressed as percentage and percentage per year, respectively. The mean values were expressed as media $\pm$ s.d.
Results
Thyroidectomy was performed in 166 females and 22 males. The mean age was 50.6 ± 14.8 years (13–85). We found 38 cases of PTM (5 M, 33 F). The mean age was 50.7 ± 13.1 years (25–78). Only one case was diagnosed before thyroidectomy. The PTM prevalence was higher in thyroid solitary nodule (40%), followed by euthyroid multinodular goitre –MNG- (28%), hypofunctioning MNG (20%), thyrogloss cyst (20%), Graves-Bassedow disease (7.7%) and toxic MNG (7.3%). The mean tumor size was 0.3 ± 0.2 mm (0.1–10). Multifocality was observed in 29.6%. One case (2.6%) has extrathyroid extension (adenopathy). Euthyroidism, hypothyroidism and hyperthyroidism were presents in 81.6%, 13.2% and 5.3%, respectively. Thyroid autoimmunity in 7.9%. The prevalence was 20.3 without sex differences (22.7 M, 20 F). The annual incidence oscilated among 2.6 and 34.2, showing a striking and progressive increase at the last years.
Conclusions
PTM is more frequent in nodular thyroid disease. Its prevalence is high, as well as its multifocality (almost 1/3), increasing at the last years. It depends on the extended indications for total thyroidectomy for benign diseases, on progress in the field of diagnostic procedures, and on the pathology examination.

P812

Thyroid cancer in a patient with ankylosing spondylitis on infliximab

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Biological agents have been successfully introduced in the therapeutics of autoimmune diseases, in particular rheumatoid arthritis and ankylosing spondylitis. However, their use has been found to increase the risk of serious infections and neoplasms. Infliximab, a neutralizing antibody to tumor necrosis factor-alpha, appears to be effective therapy in ankylosing spondylitis. Tumor necrosis factor (TNF) plays an important role in host defense and tumor growth control. Therefore, anti-TNF antibody therapies may increase the risk of serious infections and malignancies.
The aim was to describe the case of a patient with ankylosing spondylitis who developed a papillary thyroid carcinoma while being on therapy with infliximab. A female patient, aged 39 years, with ankylosing spondylitis diagnosed at the age of 19 years, was given infliximab. Four years after the initiation of therapy nodular disease of the thyroid was diagnosed, as an incidental finding in computer tomography scanning of the spine. A fine needle aspiration biopsy was performed showing a papillary thyroid carcinoma. Near total thyroidectomy was performed. On histolory a papillary thyroid carcinoma of the follicular type was diagnosed. She was given radioiodine therapy. Infliximab therapy was stopped and ankylosing spondylitis flared.
Conclusion
Treatment with anti-TNF-a biological agents in rheumatoid arthritis has been reported to increase the risk of malignancies, namely skin cancers and malignant lymphoma. Treatment with infliximab in ankylosing spondylitis has been found to increase the risk of serious infections. In the case described treatment with infliximab may have been related to the occurrence of a papillary thyroid carcinoma of the follicular type. This is the first report of thyroid cancer, which may be linked to anti-TNF-a biological agent therapy in ankylosing spondylitis.

P813

Changes in cystatin C during the treatment of subclinical hypothyroidism

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Background
Serum cystatin C is a novel marker for kidney function that has been claimed to be superior to serum creatinine. Cystatin C concentrations increased in the hyperthyroid patients and decreased in the hypothyroid patients. The cystatin C test detects kidney disease at earlier stages, before symptoms appear and creatinine levels rise. Another advantage is that, unlike creatinine, blood levels of cystatin C are less influenced by age, gender, race, or lean muscle mass, which makes it a better indicator of kidney function. This study was performed to evaluate changes in cystatin C and creatinine during the treatment of subclinical hypothyroidism (SH).
Methods
Cystatin C, creatinine, CRP and lipids were determined at the time of diagnosis of SH (TSH$>$4.2 mIU/ml with normal level of fT3 and fT4), and when TSH

returned into the normal range after treatment with levothyroxine in 35 SH women ages 35.3±9.5 years.
Results
TSH was 9.4±4.3 mU/l (reference 0.3–4.2) at diagnosis and decreased to 2.9±1.2 mU/l following treatment with levothyroxine. Cystatin C increased from 0.68±0.19 mg/l (reference 0.5–0.96) in the hypothyroid state to 0.89±0.16 mg/l when TSH normalized ($P<0.01$). Creatinine decreased from 98±11 umol/l (reference 45–115) in the hypothyroid state to 67±14 umol/l when TSH normalized ($P<0.05$). CRP levels also decreased when TSH normalized (5.2±1.1 vs 2.5±0.9 mg/l). Mean total and LDL-cholesterol levels decreased too, but not significantly.
Conclusion
Subclinical hypothyroidism has a substantial impact on cystatin C levels. In contrast to creatinine concentrations, Cystatin C levels are lower in the hypothyroid state as compared with the euthyroid state. Therefore, thyroid function has to be considered when cystatin C is used as a marker of kidney function at many diseases like diabetes, hypertension, and cardiovascular diseases.

P814

Mixed papillary, poorly differentiated and anaplastic thyroid carcinoma

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Well differentiated thyroid carcinoma is associated with prolonged survival in terms of decades and anaplastic carcinoma is associated with survival in months. Intermediate malignancy with a more guarded prognosis is also known to exist. The aim was to describe the case of a patient with a mixed papillary, poorly differentiated and anaplastic thyroid carcinoma.
A female patient, aged 73 years presented with a multinodular goiter and associated lymphadenopathy. A near total thyroidectomy was performed with lymph node dissection. On histology a diffuse thyroid carcinoma was observed. It was found to have areas of papillary differentiation, areas of insular architecture and areas of anaplastica carcinoma, multiple psammoma bodies and foci of fibrosis and calcification. Multiple neoplasmatic emboli were noted within the blood vessels and massive metastatic disease in the lymph nodes. The carcinoma was found to invade the surgical limits of dissection. A whole body scan was performed with ^{131}I which was negative. She was given 100 mCi ^{131}I. On follow up she developed metastatic disease in the area of the neck.
Multinodular goiter is known to be a risk factor for the development of thyroid cancer and in some cases the development of anaplastic carcinoma. Carcinogenesis in the thyroid is known to be a multi-step procedure. Papillary thyroid carcinoma is sometimes dedifferentiated to forms of carcinoma with less favorable prognosis. In the case described areas of papillary carcinoma were found along with areas of insular carcinoma and areas of anaplastic carcinoma. As predicted by the histology and the massive metastatic disease the clinical course of the patient was not favorable. In this extremely rare case of thyroid carcinoma the multi-step procedure of carcinogenesis is characteristically depicted in the histological findings.

P815

Prevalence of primary hypothyroidism in an obese population

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A positive association between thyrotropin (TSH) secretion and body-mass index (BMI) was previously reported. There are many studies concerning thyroid function in obesity, and some of them describe higher TSH levels in obese subjects.
Evaluation of the hypothyroidism prevalence in a sample of obese women in their first obesity appointment at Hospital São João.
Anthropometric variables and serum levels of TSH, free triiodothyronine (FT3) and free thyroxine (FT4) were evaluated. They were asked if they were undergoing levothyroxine (LT4) replacement therapy. TSH values between 0.35 and 4.94 ng/dl were consider euthyroidism.
A sample of 257 women having a mean age of 40.9±11.2 years old and a mean BMI of 44.6±7.1 kg/m^2 was evaluated. We found a primary hypothyroidism prevalence of 13.2%. The prevalence of diagnosed hypothyroidism undergoing levothyroxine replacement therapy was 5.8% (i.e. 15 patients). From these 5.8%, 86.7% were well-controlled, while 13.3% maintained high levels of TSH under therapy. In the remaining 94.2% (i.e. 242 patients), a prevalence of 7% hypothyroidism, 92.6% euthyroidism and 0.4% hyperthyroidism was found. From the above-mentioned 7% of patients with hypothyroidism, a prevalence of subclinical hypothyroidism was found to be 68.4%.
In the same sample, the mean age of patients with hypothyroidism was 39.3±11.6 years old with a mean BMI of 47.9±11.6 kg/m^2, and a mean waist circumference of 128.4±18 cm, while those having euthyroidism revealed a mean age of 41.1±11.1 years old, a mean BMI of 44.3±6.8 kg/m^2 and a mean waist circumference of 120.6±13.4 cm. The only patient with hyperthyroidism was 20 years old and revealed a BMI of 60.2 kg/m^2.
From this study, the occurrence of a low hypothyroidism prevalence in the analysed sample could be observed. Furthermore, the analysed female patients with hypothyroidism revealed higher BMI and waist circumference and a lower age average than those having normothyroidism.

P816

Thyroid autoimmunity and assisted reproductive technology (ART)

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About 5–10% of fertile women have anti-thyroid antibodies (AAT); however this prevalence is higher in infertile women. Few studies have been focused so far on the role of thyroid autoimmunity in the outcome of ART. Aims of our studies have therefore been: a) evaluate prevalence of thyroid antibodies in infertile women before ART; b) investigate whether AAT are more prevalent in a specific subset of infertile women; c) investigate the possible influence of AAT or thyroid dysfunction on the outcome of ART.
About 105 women (mean age 35.7+4.7) have been investigated; 11 subjects have been excluded from the study because of known thyroid diseases. The remaining 95 women have been divided according to the cause of infertility; 34% were idiopathic, 26.6% had a male factor, 6.4% had ovulatory disorders, 10% had endometriosis and 17.6% had tubal factor. TSH, FT4, Tg-Ab, TPO-Ab, TSHr-Ab (both stimulating and blocking) have been assayed before ovarian stimulation. AAT have been detected in 23 (24.4%) women, all of them being euthyroid except 1 subclinical hyperthyroid and 1 subclinical hypothyroid. The highest prevalence of AAT (27%) have been found in women with tubal factor infertility. Out of the 94 women, 85 have undergone ART (9 being non-responders to ovarian stimulation) and pregnancy was obtained in 23 cases; in 3 cases, however, precocious abortion has been observed. 6 out of these 23 were AAT positive (26%, 5 deliveries, 1 abortion). Amongst the 62 non-pregnant women, 15 were AAT positive (24.2%). TSH was not significantly different (1.6+0.95 mUI/l vs 1.8+1.04 mUI/l for pregnant and non-pregnant women respectively).
Nine women were positive for TSHr-Ab (in 7 cases without contemporary TPO- or Tg-Ab) 2 of them becoming pregnant. According to TSH value 70 (82.3%) women had a value below 2.5 mUI/l; 21 (30%) of them became pregnant but 2 had precocious abortion. Fifteen (17.7%) women had TSH value above 2.5 mUI/l (but still within the normal range); 3 (20%) became pregnant but 1 had precocious abortion.
Our data suggest; a) higher prevalence of AAT in infertile women; b) AAT were more frequently associated to tubal factor infertility; c) more cases are needed in order to draw final conclusion about the possible interference of within-the-normal-range TSH value above 2.5 mUI/l with the positive outcome of ART.

P817

Five new mutations leading to partial deficiency of thyroxine-binding globulin

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Introduction
Thyroxine-binding globulin (TBG) is the main thyroid hormone transport protein in blood. So far, 26 TBG deficiency mutations have been reported, 7 of them leading to partial TBG deficiency. We examined T4 binding capacity and the TBG gene in patients with normal free T4 (fT4) and reduced total T4 (TT4). We present and characterize five new partial TBG deficiency mutations.
Methods
Automated chemiluminescence immunoassays were used for the determination of TSH, fT4, TT4 and TBG. In case of TBG deficiency, the extent of deficiency was determined by a TBG-specific T4-binding assay. Direct DNA sequencing of the TBG gene (exons 1-4 and the non-coding exon 0) was used to identify mutations in the propositi. To characterize the mutations, they were introduced into a TBG expression vector, which was then transfected into HepG2 cells. After 48 h, T4-binding capacity, representing TBG concentration, was measured in the media.
Results
T4 binding capacity in media of cells transfected with a normal TBG-vector was set as 100%. T4 binding capacity in media of cells transfected with an empty vector as a negative control was 2.6%. Five TBG mutations lead to a severe reduction of T4 binding capacity, representing partial TBG deficiency: R35Q: 11.4%; S52R: 12.4%; N112L: 12.0%; R381G: 4.8%; S382R: 10.5%.
Conclusion
Mutations in the TBG gene can lead to TBG deficiency. We report 5 new partial TBG deficiency mutations and experimentally characterized their T4 binding capacity.

P818

Clinical evaluation of 3rd generation assay for thyrotropin receptor autoantibodies in autoimmune thyroid diseases

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Recently a new procedure for measuring serum TSH receptor (TSHR) autoantibody (TRAb) was reported in which the autoantibodies inhibit binding of a human monoclonal thyroid stimulating antibody M22 (labeled with biotin) to TSHR-coated ELISA plate wells. This assay was termed 3rd generation TRAb assay. The aim of the present study was to clinically evaluate the TRAb 3rd generation assay in comparison with the 2nd generation TRAb assay (TRAb-2nd) based on the recombinant human TSH-receptor.
Altogether, 158 patients were analyzed, of whom 84 patients suffered from Graves' disease (GD), 34 patients had Hashimoto's thyroiditis (HT) and 40 patients had euthyroid nodular thyroid disease (NTD) without signs of autoimmunity. TRAb measurements were performed according to the manufacturer's instructions. The TRAb-2nd assay revealed a high sensitivity and specificity as 81 of 84 (96.4%) GD patients were positive. One GD patient had TRAb values within the grey zone (1.0–1.5 IU/l). All patients with HT and NTD were negative except 6 (17.6%) and 3 (7.5%) cases with TRAb values within the grey zone. On the basis of the TRAb-3rd assay, 77/84 (91.6%) GD patients were TRAb positive. One patient (2.9%) with HT and three cases (7.5%) with NTD were also TRAb positive. The sensitivity of the second and third generation assays were 93% and 84%, while the specificity were 100% and 96.2%, respectively. There was a close correlation ($r=0.821$, $P<0.0001$) between TRAb-3rd and TRAb-2nd assays in 84 patients with GD. Problematically, the interassay coefficient of variation was 28.2% using the recommended cut-off limit of 1 IU/l.
Our results indicate that the 3rd generations TRAb assay has a lower sensitivity and specificity compared to the second generation human TSH receptor based TRAb assay.

P819

First rapid and automated immunoassay for TSH receptor antibodies

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Hyperthyroidism in Graves' disease is due to TSH receptor (TSHR) autoantibodies (TRAb) directed against the TSH binding pocket. Detection of TRAb in clinical rou-tine is performed by 2nd gen assays using human or porcine TSHR. Such TSH bin- ding inhibition immunoglobulin (TBII) assays determine the ability of TRAb to inhi- bit the binding of labelled TSH to immobilized TSHR. TRAb are present in low con- centrations and mostly recognize conformational epitopes. The conformational structure of the TSHR is sensitive to thermal stress. Incubation times of current as- says must be for a minimum of 2 h to obtain good sensitivity. Due to these tech- nical difficulties no rapid automated TRAb assay could be developed so far.
We report on the 1st automated TRAb assay on the Elecsys/ cobas e immuno-assay platform. TRAb inhibit the binding of a human monoclonal thyroid stimulating autoantibody (M22; labelled with a ruthenium complex) to preformed immuno- complexes based on aggregates of solubilised multiple porcine TSHR (pTSHR) and a mouse monoclonal capture antibody (4E31 IgG; labelled with biotin). 4E31 does not interfere with the binding of TRAb and M22 to the pTSHR. The lyophili- zed immunocomplexes are stabilized after reconstitution by chemical chaperones. The assay uses a delayed competitive test principle. Total assay time is 3×9 min only. Firstly, sample TRAb – if present – bind to the multiple TSHR binding sites. Secondly, TRAb are allowed to interact with the TSHR further. Thirdly, labelled M22 and streptavidin-coated microparticles are added. M22 binds to still free bin- ding sites. Immunocomplexes are bound to the solid phase via interaction of biotin and streptavidin. Assay calibration is against the NIBSC 90/672. Its measuring range extends from 0.3–40 IU/l. The functional sensitivity is $\sim$0.9 IU/l.
The automated Elecsys Anti-TSHR correlates well with current TBII assays and provides clinical performance and total precision which will meet customer needs.

P820

Visual evoked potentials in children with hypothyroidism

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The clinical picture of hypothyroidism is well described. It is well known that thyroid hormones are very important to development and maturation of the central nervous system. They have influence on the synthesis of proteins and production of enzymes and myelin.
Myelin synthesis is an important factor in determining the speed of impulse transmission along complex polysynaptic pathways, such as those mediating the evoked potentials.
Visual evoked potentials are reliable and objective method for measuring the function of visual pathway conduction. Visual Evoked Potentials (VEP) were performed in 40 children. We studied 26 girls and 14 boys. The flash stimulation with frequency of 1 Hz was used.
At the time of the present study patients were euthyroid with no clinical signs of disease.
The latencies to the peak P100 were measured. We compared the results with normal control group of 35 children (21 girls and 14 boys). The recording of VEP is a sensitive technique for detection of subclinical lesions of the visual system.

P821

Iodothyronine antibodies are not correlated with prevalence or prognosis of non-thyroidal illness syndrome

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Patients suffering from critical illness usually exhibit a characteristic functional state of thyrotropic feedback control that is referred to as non-thyroidal illness syndrome (NTIS). Today, there are still important questions unsolved, e.g. regarding the interdependence of immunological and endocrine functions in critical illness.
Therefore, in the context of the prospective AQUA FONTIS study we investigated 164 patients that were treated in medical, surgical and heart surgical intensive care units (ICUs) of the Bergmannsheil university hospitals in Bochum, Germany. Here, we examined the titres of antibodies reacting with thyroxine (T4-ab) and triiodothyronine (T3-ab) that were determined 24 h after admission to

the ICU, and investigated their correlation with parameters of thyroid homeostasis, length of stay in hospital (LOSIH) or ICU (LOSICU) and survival of patients.
All patients exhibited Gaussian distributed antibody titres that were in the normal range for healthy volunteers. Moreover, the titres didn't correlate with functional characteristics like thyrotroph thyroid hormone sensitivity index (TTSI) as a parameter for the central component of NTIS, or sum activity of 5′-deiodinase as a marker for impaired deiodination. Again, antibody titres didn't exhibit correlation with survival, LOSIH or LOSICU, in the latter two cases even if only the subgroup of surviving patients was considered.
In conclusion, despite of immunological activation, e.g. in case of sepsis, antibodies directed against T3 or T4 are not elevated among critically ill patients nor do they play a role in the prognosis of the disease.

P822

No association between $BRAF^{V600E}$ and metalloproteinase activity in anaplastic thyroid cell lines

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A strong evidence suggests the role of $BRAF^{V600E}$ mutation in determining the aggressiveness of thyroid cancer. To detect how the association of BRAF mutation could influence the aggressiveness of particular subtypes of thyroid tumors, we analysed a possible correlation between $BRAF^{V600E}$ and metalloproteinase (MMPs) activity, as its enhanced production could be an important determinant of tumor invasion. For this purpose, we investigated the activity and the expression of different MMPs in anaplastic thyroid cell lines, such as FRO, ARO and KAT-18, by means of zymography and real time-polymerase chain reaction (RT-PCR). FRO and ARO cell lines are homozygote and heterozygote respectively for $BRAF^{V600E}$, while KAT-18 cells are negative for BRAF mutation.
Analysis of the enzymatic activity in the different cell supernatants showed that the activity of MMP-2 and MMP-9 was absent in ARO cell line and low in FRO cell lines. In KAT-18 we have found a higher MMP-2 activity, instead MMP-9 resulted absent.
Real-time PCR revealed that MMP3 and MMP-2 were higher in KAT-18 than FRO, while it was absent in ARO cells. The MMP-9 was absent in ARO and KAT-18 and low expressed in FRO. In summary, $BRAF^{WT/MUT}$ ARO cell line was negative for all MMPs, while $BRAF^{MUT/MUT}$ FRO was faintly positive. $BRAF^{WT/WT}$ KAT-18 cell line was strongly positive for MMP2 and MMP3 and negative for MMP9.
Our results show that no complete association between $BRAF^{V600E}$ and MMPs activity seems to exist. Our findings could be explained by the fact that not all the MMPs genes have an AP-1 site in promoter region regulated by BRAF-dependent ERK pathway. Several other transcription factors, such as ETS, NF-kB, and STAT, may modulate differently the expression of each MMP gene. In conclusion, BRAF could be involved in tumor progression through other mechanisms independently from MMPs pathway.

P823

Analysis of selected adipocytokines in children with autoimmune thyroid diseases

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Leptin, adiponectin and resistin play an essential role in regulation of body mass, which is controlled by thyroid hormones levels.
The aim of the study was to evaluate leptin, adiponectin and resistin levels in young patients with Graves' disease and in children with Hashimoto's thyroiditis. The study group formed 78 patients suffering from Graves' disease (30 girls and 2 boys; aged from 6 to 21- mean 15.2 years) and Hashimoto's thyroiditis (30 girls and 3 boys; aged from 9 to 18- mean 14.5 years). The control group consisted of children with struma nodosa- 11 girls and 2 boys; aged from 9 to 18 -mean 14.6 years. In all patients were performed leptin, adiponectin and resistin levels – ELISA' method (R&D System, USA).
In children with Graves' disease we found significantly higher levels of leptin compared to group with Hashimoto's thyroiditis (12.64 ng/ml vs 7.67 ng/ml, $P<0.001$). Adiponectin level was higher in children suffering from Graves' disease compared to group with control and Hashimoto's thyroiditis (12.33 μg/ml vs 11.7 μg/ml, $P<0.05$; vs 11.21 μg/ml, $P<0.05$). Resistin level was higher in group of hypothyroid than hyperthyroid (14.199 ng/ml vs 12.42 ng/ml, $P<0.01$). We conclude that thyroid dysfunction plays significant affect on adipocytokine concentrations.

P824

Moderate hyperhomocysteinemia in hypothyroidism

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Moderately elevated plasma homocysteine levels (>12 μmol/l) are highly prevalent in the general population and are associated with an increased risk for cardiovascular disease. Homocysteine is an amino acid produced during catabolism of the essential amino acid methionine. Metabolism of homocysteine requires an adequate supply of folic acid, vitamin B12 and vitamin B6.
Moderate hyperhomocysteinemia may be caused by a variety of factors: chronic disorders (hypothyroidism, diabetes mellitus, systemic lupus erythematosus, chronic renal failure), nutritional deficiencies (folic acid, vitamin B12, vitamin B6), drugs (methotrexate, anticonvulsant agents, cyclosporine), postmenopausal status, smokers, enzyme deficiencies.
Measurement of plasma homocysteine was performed by ELISA method, during the fasting state.
Hypothyroidism is associated with increased cardiovascular morbidity, which might be explained by the atherogenic lipid profile and/or hyperhomocysteinemia.
We studied plasma homocysteine and folate levels in a group of 25 patients with primary hypothyroidism, before and during levothyroxine treatment. After achieving euthyroidism, plasma homocysteine and folate were also measured before and after three months of daily folate supplimentation. In some of our patients with plasma homocysteine lower than 10 μmol/l during levothyroxine therapy, a significant decrease of plasma homocisteine levels was achieved after the folate supplementation.

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